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RELATIVE YIELDS OF INBRED LINES AND F_1 HYBRIDS OF TOMATO

LEROY POWERS

Introduction

The relative merits of inbred lines and F_1 hybrids for increasing yield of tomatoes is one of the most important problems confronting tomato breeders today. POWERS (13, 14), POWERS and LYON (16), and LYON (12) have published data on the F_1 hybrids, F_2 generations, first generation backcrosses to both parents, and the parents which showed that the use of F_1 hybrids for the commercial production of tomatoes has considerable promise. The desirability of using genetically diverse parents as a basis for producing the inbred lines to be used as parents for the F_1 hybrids was given special emphasis. The following crosses were used in establishing the inbred lines: Danmark × Johannisfeuer, Johannisfeuer × Bonny Best, Danmark × Red Currant, Johannisfeuer × Red Currant, Danmark × Ponderosa, and Ponderosa × Porter. By 1941 and 1942, some of the inbred lines were sufficiently close to homozygosity to justify further studies concerning the relative merits of these inbred lines in F_1 hybrid combinations. The literature dealing with the merits of F_1 hybrids derived from crossing existing commercial varieties and the problems involved in the production of first-generation hybrid tomato seed for commercial planting has been discussed by BARRONS and LUCAS (3) and BARRONS (2). The primary purpose of this study is to evaluate the F_1 hybrids derived from inbred lines produced from parents of wide genetic diversity.

Of particular importance in attaining this objective are: the partitioning of the different characters into their components; studying the range in variation of these characters; determining to what extent the desired expression of a character or combination of characters can be attained in the inbred lines and F_1 hybrids; and finally, determining the combining ability of the various inbred lines as regards the characters under consideration. Heterosis and dominance are important in determining the combining ability or extent of expression of a character in the F_1 hybrids. Some of the genetic factors regulating the ease or difficulty with which the genes differentiating the various characters can be recombined in individual F_1 hybrid populations and inbred lines are the number of gene pairs differentiating the character or characters under consideration, the linkage relations of these genes, and pleiotropy.

The climatic conditions at Cheyenne, Wyoming, are those typical of the higher Great Plains region. The elevation above sea level of the Cheyenne Horticultural Field Station is 6300 feet. The average time between the last killing frost in the spring and the first in the fall is 141 days. The average annual precipitation is 15.82 inches. However, additional moisture as needed was supplied by irrigation to the experimental plots from which the data for this study were taken. For these climatic data and further details concerning the climatic conditions existing at Cheyenne, see KINCER (11).

Material and methods

The material grown during 1941 consisted of the inbred lines Red Currant, Johannisfeuer, Danmark, their possible F_1 hybrids, and three inbred lines derived from the cross Johannisfeuer × Red Currant. The material grown in 1942 consisted of ten inbred lines and all the different hybrids possible among them. Eight of the inbred lines were derived by continued selection of individual plants from the later generations of the hybrids previously reported on by POWERS (14), POWERS and LYON (16), and LYON (12); the other two were Bounty and Danmark, these being used because they were the most outstanding commercial varieties tested at the Cheyenne station, where the studies were conducted. This investigation is in reality a continuation and expansion of the studies reported in the preceding citations.

The experimental design consisted of ten randomized complete blocks, one plot of each variate being grown per block. Each plot was composed of twelve plants 4 feet apart within rows and the rows spaced 4 feet apart. Since the interest in this study is in the comparisons between the means of the F_1 hybrids and inbred lines, the plot instead of the plant was used as the unit for reducing the data. Then, for each parent and generation the standard errors were estimated for within the F_1 hybrids and inbred lines on the basis of the ten-plot means, these means having been derived from the individual plant data for the respective plots. In all tables the standard errors of the means are given. As in the previous work, cited above, a generalized error was not used; instead, a standard error was calculated for each variate on the basis of the ten-plot replicates of that variate. The data for weight of ripe fruit per plant, number of ripe fruit, and weight of individual fruit were taken from two plants of each plot. These two plants were chosen at random by means of TIPPETT's tables (19). The data for all other characters were taken from all plants of each plot.

The method employed in studying the characters was to partition them into their different components:

- Yield of ripe fruit
  - Number of ripe fruit
    - Days from seeding to first fruit ripe (earliness)
      - Days from seeding to first bloom
      - Days from first bloom to first fruit set
      - Days from first fruit set to first fruit ripe
    - Spread of plant
    - Height of plant
  - Weight of individual fruit
    - Number of locules
    - Weight per locule

Yield per plant is the weight in grams of the fruit that ripened during the growing season. Spread and height of plant are expressed in centimeters; height is the distance from the ground level at the base of the plant to the top, and spread is the diameter measured through the main stem as the center. Days was the unit employed for measuring earliness of maturity and its different components. The details of how the earliness of maturity measurements are taken are given in a previous article (16). Weight per fruit in grams was obtained by weighing the ripe fruit and dividing by the number that ripened: Number of locules was determined from transverse sections of the fruit. Weight per locule was obtained by dividing weight of fruit by number of locules. The results from the studies on the interrelations of weight per fruit,

number of locules, and weight per locule show that weight per locule (as determined) is a character of considerable importance in breeding larger-sized fruits. Data supporting this conclusion are given in the section on the relation between weight of individual fruit, number of locules per fruit, and weight per locule (p. 265).

Tables of means are given for all characters except number of days from seeding to first bloom, number of days from first bloom to first fruit set, and height of plant.

It should be noted that the variation of yield, weight of individual fruits, and number of days from seeding to first fruit ripe is completely accounted for by the multiple regression of these characters on their respective components; that is, R^2 multiplied by 100 should equal 100%, if the precise description of the concomitant variation of the quantities has been discovered and the appropriate transformation used in calculating the data. Hence, the closeness with which R^2 times 100 approaches the value 100% is a measure of the agreement between the assumed and the actual nature of the covariation of the variates; that is, if the proper scale of measurement (**21, 5**) has been used in determining the R^2 and r^2 values.

Also, it should be noted that r^2 times 100 is the percentage of the variation (either sums of squares, Sy^2 or Sx^2, or variance, Vy or Vx) that can be accounted for by covariation; that is, by the regression of one character on the other. For definition of terminology, use of symbols, and methods of reducing the data, see SNEDECOR (**17**).

The method of grouping the means may be seen in table 1. The inbreds may be grouped into four main classes on the basis of their behavior in crosses: (*a*) The first class is composed of 4101, 4102, and 4105; (*b*) the second class of 4103 and 4104; (*c*) the third class of 4106, 4107, and 4108; and (*d*) the fourth class of 4109 and 4110. In the tables the data for the inbreds and hybrids among inbreds within each group are blocked off by bold-faced lines, while interclass hybrids lie outside these lines. The interclass hybrids between the inbred lines are further delimited by broken lines.

The use of the terms heterosis and dominance throughout this paper is consistent with the information on these phenomena reported by POWERS (**14, 15**).

Experimental results

YIELD OF RIPE FRUIT PER PLANT

The average weights of ripe fruits per plant harvested during the growing season of 1942 are given in table 1. The grand means of the inbred lines and those of the hybrids are listed in the last row of this table. The F_1 hybrids are far superior to the inbred lines in yielding ability, producing 59% more ripe fruit by weight. That the average yield of any inbred line is surpassed by the grand mean of its respective hybrids can be seen by comparing the figure at the foot of each column with the figure in the same row of the last column. Only six of the forty-five hybrids failed to show heterosis.

Of the four classes depicted by the solid demarcation lines, no. 3 is the only one in which intraclass heterosis is questionable, indicating that these inbred lines of class 3 have inferior combining ability. That they lack superior combining ability with some other inbred lines as well is shown by the fact that five of the six hybrids not showing heterosis had these inbred lines as parents.

The outstanding hybrid combinations in regard to yield are 4109 × 1410 and

4102 × 4110. They yielded approximately three times as much as Danmark, the variety recommended for commercial production by BABB and KRAUS (1). Of the hybrids having larger-sized fruit, 4101 × 4103 was the highest producer, yielding 1827 gm. of ripe fruit per plant as compared with 828 gm. for Danmark. These data indicate the striking increase in yield attainable by utilizing heterosis.

The inquiry should not end with the discovery of the high yielding combinations; rather, the origin of these inbred lines should be considered in relation to their ability to produce high yielding hybrids. The inbred lines fall into two groups as to parentage: 4101, 4102, 4105, and 4106 are of *Lycopersicon esculentum* Mill. and the other six have resulted from crosses involving *L. esculentum* and *L. pimpinellifolium* (Jusl.) Mill. (Red Currant). From the data of table 1 it can be seen that none of the F_1 hybrids involving inbred lines derived solely from *L. esculentum* possessed special yielding ability. All the high producing F_1 hybrids have at least one parent derived from crosses between *L. esculentum* and *L. pimpinellifolium*. Again these findings emphasize the importance of developing and selecting the inbred lines in such a way that they have as wide a diversity of genes initiating those substances and reactions responsible for the development of the character sought as it is possible to attain.

The outstanding inbred lines in F_1 hybrid combinations (table 1) are themselves the three highest yielders—4110, 4109, and 4103. Just how far the breeder can go in employing yield of inbred lines to discover those having exceptional combining ability cannot be deduced from these data. Extensive investigations on heterosis and dominance involving numerous and diverse breeding stocks are required before conclusions of general application to combining ability can be drawn.

NUMBER OF RIPE FRUIT PER PLANT

The means for number of ripe fruit per plant are listed in table 2. The data show that the grand mean of the inbred lines and the grand mean of the F_1 hybrids are not greatly different and that the hybrids for the first eight inbred lines (4101 to 4108, inclusive) average a greater number of ripe fruit per plant than their respective inbred lines, whereas the reverse is true for similar comparisons involving 4109 and 4110. Obviously, the behavior of the hybrids is not the same, and therefore the data of this table must be considered in more detail.

The intraclass hybrids of classes 1, 2, and 4 show heterosis, whereas those of class 3 do not. Also, the interclass hybrids between classes 2 and 3 have a tendency toward a slight degree of heterosis. The interclass hybrids of 1 and 2 and of 1 and 3 show partial dominance of greater number of fruit per plant, the degree of expression approximating rather closely that of complete dominance. The interclass hybrids involving classes 2 and 4, and those involving classes 3 and 4, show partial dominance for greater number of fruit; but the degree of expression is only a little above that of no dominance. On the whole, the interclass hybrids involving classes 1 and 4 show partial dominance for fewer fruit per plant. Again, the degree of partial dominance is not marked, being not greatly different from no dominance. These data show that in the F_1 hybrids the expression of number of ripe fruit per plant ranges from partial dominance of fewer fruit to heterosis for more fruit per plant. This statement has added importance when one considers that the range in this material is from 2.9 to 183.2 ripe fruit per plant.

TABLE 1

MEANS FOR YIELD (IN GRAMS) OF RIPE FRUITS PER PLANT HARVESTED DURING GROWING SEASON*

Grand means

TABLE 1

MEANS FOR YIELD (IN GRAMS) OF RIPE FRUITS PER PLANT HARVESTED DURING GROWING SEASON*

	Variety or inbred line	Bounty 4101	[(D×J)×D] S_6 4102	[(J×BB)×BB] S_5 4105	[(D×RC)×D] S_5 4103	[(D×RC)×D] S_5 4104	Danmark 4106	[(D×RC)×D] S_6 4107	[(D×RC)×D] S_6 4108	(J×RC) F_9 4109	(J×RC) F_8 4110	Grand means for hybrids
1	Bounty, 4101	513±39	934±182	675± 98	1827±196	1216±138	1036±168	907±131	964±128	1653±168	2310±186	1280±53
	[(D×J)×D] S_6, 4102		607± 86	882±142	1367±148	1165±132	767±118†	871±127	934±140	2059± 81	2428±150	1267±46
	[(J×BB)×BB] S_5, 4105			332± 64	1352±138	890± 93	798± 79†	598± 83†	695± 75†	2004± 33	1831±107†	1081±33
2	[(D×RC)×D] S_5, 4103				1066±159	1459±136	1433±127	1551±194	1279±128	1842±220	2266±127	1597±54
	[(D×RC)×D] S_5, 4104					808±114	1186±111	1195±166	1208± 97	1537± 71	2201±195	1340±44
3	Danmark, 4106						828±108	929±169	860± 92	1969±119	2144±192	1236±45
	[(D×RC)×D] S_6, 4107							801±111	785±138†	1852±122	1942± 91	1181±47
	[(D×RC)×D] S_6, 4108								857±108	1921±154	2082±136	1192±41
4	(J×RC) F_9, 4109									1364±151	2876±177	1968±46
	(J×RC) F_8, 4110										1868±149	2231±52
	Grand mean yields of inbred lines and of hybrids, respectively										904± 36	1437±15

TABLE 2

MEANS FOR NUMBER OF RIPE FRUIT PER PLANT*

Variety or inbred line	Bounty 4101	[(D×J)×D] S_6 4102	[(J×BB)×BB] S_5 4105	[(D×RC)×D] S_5 4103	[(D×RC)×D] S_5 4104	Danmark 4106	[(D×RC)×D] S_6 4107	[(D×RC)×D] S_6 4108	(J×RC) F_9 4109	(J×RC) F_8 4110	Grand means for hybrids
Bounty, 4101	4.3±0.32	8.2±1.40	6.0±1.01	20.5±2.27	14.4±1.99	10.5±1.69	8.8±1.32	8.9±1.29	54.0± 5.26	55.9± 5.92	20.8±1.00
[(D×J)×D] S_6, 4102		4.4±0.69	8.2±1.41	16.9±1.88	14.7±1.53	8.7±1.54	9.1±1.26	10.3±1.58	51.9± 2.24	44.5± 2.52	19.2±0.58
[(J×BB)×BB] S_5, 4105			2.9±0.62	18.8±2.19	11.3±1.21	7.4±0.79	6.3±0.78	6.8±0.70	66.5± 3.34	43.4± 2.34	19.4±0.58
[(D×RC)×D] S_5, 4103				19.5±2.94	28.6±3.82	20.1±2.31	22.2±2.90	18.9±2.17	85.4± 8.33	87.1± 6.95	35.4±1.42
[(D×RC)×D] S_5, 4104					15.5±2.48	17.0±1.66	19.2±2.55	17.6±1.50	79.8± 4.71	88.6± 7.93	32.4±1.21
Danmark, 4106						8.8±1.08	10.3±1.90	9.7±1.13	73.3± 4.45	65.7± 5.85	24.7±0.95
[(D×RC)×D] S_6, 4107							9.4±1.54	9.4±1.47	68.0± 3.18	59.1± 4.13	23.6±0.80
[(D×RC)×D] S_6, 4108								10.7±1.21	75.1± 5.61	62.5± 4.75	24.4±0.92
(J×RC) F_9, 4109									118.3±12.91	183.2±13.32	81.9±2.15
(J×RC) F_8, 4110										109.1±11.34	76.7±2.25
Grand means for number of ripe fruit per plant of inbred lines and of hybrids, respectively										30.3± 1.78	35.9±0.42

* Symbols: D, Danmark; J, Johannisfeuer; RC, Red Currant; BB, Bonny Best.

† Average yield lower than that of highest yielding parent.

TABLE 3

MEANS FOR WEIGHT (IN GRAMS) OF INDIVIDUAL FRUIT*

Variety or inbred line	Bounty 4101	[(D×J)×D] S_6 4102	[(J×BB)×BB] S_5 4105	[(D×RC)×D] S_5 4103	[(D×RC)×D] S_5 4104	Danmark 4106	[(D×RC)×D] S_6 4107	[(D×RC)×D] S_6 4108	(J×RC) F_9 4109	(J×RC) F_8 4110	Grand means for hybrids
Bounty, 4101	119±6.49	114± 4.91	112±7.37	89±3.66	85±7.13	99± 3.79	103±6.47	108±4.65	31±1.11	41±2.14	87±1.67
[(D×J)×D] S_6, 4102	.....	138±12.81	108±4.66	81±4.06	80±1.14	88±13.18	96±4.36	91±4.43	40±1.15	55±2.93	84±1.88
[(J×BB)×BB] S_5, 4105	.....	.....	114±8.49	72±3.14	79±2.82	109± 7.31	96±7.27	103±3.99	30±1.26	42±2.28	83±1.66
[(D×RC)×D] S_5, 4103	.....	.....	.....	55±2.74	51±2.87	71± 3.92	70±3.97	68±4.82	22±1.05	26±1.76	61±1.15
[(D×RC)×D] S_5, 4104	.....	.....	.....	.....	52±2.74	70± 4.12	62±2.21	69±2.25	19±1.16	25±0.62	60±1.09
Danmark, 4106	.....	.....	.....	.....	.....	94± 6.39	91±5.06	89±4.05	27±1.47	33±1.22	75±1.99
[(D×RC)×D] S_6, 4107	.....	.....	.....	.....	.....	.....	85±4.17	83±3.26	27±1.03	33±1.32	73±1.46
[(D×RC)×D] S_6, 4108	.....	.....	.....	.....	.....	.....	.....	80±3.45	26±0.80	33±1.28	74±1.19
(J×RC) F_9, 4109	.....	.....	.....	.....	.....	.....	.....	.....	12±0.50	16±0.68	26±0.37
(J×RC) F_8, 4110	.....	.....	.....	.....	.....	.....	.....	.....	.....	17±0.58	34±0.58
Grand means for inbred lines and hybrids, respectively										77±1.91	66±0.44

TABLE 4

MEANS FOR NUMBER OF DAYS FROM SEEDING TO FIRST FRUIT RIPE*

Variety or inbred line	Bounty 4101	[(D×J)×D] S_6 4102	[(J×BB)×BB] S_5 4105	[(D×RC)×D] S_5 4103	[(D×RC)×D] S_5 4104	Danmark 4106	[(D×RC)×D] S_6 4107	[(D×RC)×D] S_6 4108	(J×RC) F_9 4109	(J×RC) F_8 4110	Grand means for hybrids
Bounty, 4101	142±0.596	137±0.792	138±0.537	134±0.433	135±0.629	136±0.737	137±0.960	137±0.746	126±0.543	128±0.879	134±0.238
[(D×J)×D] S_6, 4102	.....	141±1.334	135±0.912	132±0.795	132±0.539	138±1.097	135±0.803	135±0.957	124±0.800	126±0.809	133± .282
[(J×BB)×BB] S_5, 4105	.....	.....	142±1.130	134±0.365	135±0.847	137±0.611	137±0.883	136±0.516	123±0.763	126±0.448	133± .227
[(D×RC)×D] S_5, 4103	.....	.....	.....	134±0.772	128±1.012	133±0.989	133±0.596	136±0.733	123±0 775	126±0.859	131± .253
[(D×RC)×D] S_5, 4104	.....	.....	.....	.....	134±0.632	134±1.120	134±0.879	135±0.442	122±0.482	122±0.539	131± .253
Danmark, 4106	.....	.....	.....	.....	.....	137±1.159	136±1.033	136±0.792	121±1.521	125±0.646	133± .329
[(D×RC)×D] S_6, 4107	.....	.....	.....	.....	.....	.....	137±0.680	136±0.772	121±0.646	126±0.554	133± .269
[(D×RC)×D] S_6, 4108	.....	.....	.....	.....	.....	.....	.....	136±0.554	122±1.218	128±0.778	133± .267
(J×RC) F_9, 4109	.....	.....	.....	.....	.....	.....	.....	.....	124±0.841	118±0.476	122± .290
(J×RC) F_8, 4110	.....	.....	.....	.....	.....	.....	.....	.....	.....	124±1.044	125±0.228
Grand means for inbred lines and hybrids, respectively										135±0.288	131±0.084

* Symbols: D, Danmark; J, Johannisfeuer; RC, Red Currant; BB, Bonny Best.

TABLE 5

MEANS FOR NUMBER OF DAYS FROM FIRST FRUIT SET TO FIRST FRUIT RIPE*

[(D×J)×D] S_1, 4111										135±0.288	131±0.084

* Symbols: D, Danmark; J, Johannisfeuer; RC, Red Currant; BB, Bonny Best.

TABLE 5

MEANS FOR NUMBER OF DAYS FROM FIRST FRUIT SET TO FIRST FRUIT RIPE*

Variety or inbred line	Bounty 4101	[(D×J)×D] S_6 4102	[(J×BB)×BB] S_5 4105	[(D×RC)×D] S_3 4103	[(D×RC)×D] S_5 4104	Danmark 4106	[(D×RC)×D] S_6 4107	[(D×RC)×D] S_6 4108	(J×RC) F_9 4109	(J×RC) F_8 4110	Grand means for hybrids
Bounty, 4101	54±0.471	52±0.327	50±0.221	49±0.327	50±0.233	53±0.504	52±0.267	51±0.340	43±0.213	45±0.379	49±0.108
[(D×J)×D] S_6, 4102		51±0.512	50±0.367	48±0.394	49± .416	53± .447	51± .611	51± .494	43± .559	43± .367	49± .150
[(J×BB)×BB] S_5, 4105			51±0.277	47±0.340	50± .428	51± .221	50± .379	50± .291	41± .277	42± .291	48± .107
[(D×RC)×D] S_3, 4103				46±0.300	46± .200	50± .563	49± .314	50± .327	41± .327	43± .423	47± .123
[(D×RC)×D] S_5, 4104					48±0.537	51± .407	50± .335	50± .249	40± .499	45± .349	48± .119
Danmark, 4106						53±0.433	52± .365	52± .180	45± .482	45± .260	50± .134
[(D×RC)×D] S_6, 4107							51±0.348	51± .473	43± .300	45± .267	49± .128
[(D×RC)×D] S_6, 4108								51±0.427	43± .422	45± .396	49± .122
(J×RC) F_9, 4109									38±0.458	40± .482	42± .137
(J×RC) F_8, 4110										42± .359	44± .121
Grand means for inbred lines and hybrids, respectively										48±0.133	48±0.040

TABLE 6

MEANS (IN CENTIMETERS) FOR SPREAD OF PLANT*

Variety or inbred line	Bounty 4101	[(D×J)×D] S_6 4102	[(J×BB)×BB] S_5 4105	[(D×RC)×D] S_3 4103	[(D×RC)×D] S_5 4104	Danmark 4106	[(D×RC)×D] S_6 4107	[(D×RC)×D] S_6 4108	(J×RC) F_9 4109	(J×RC) F_8 4110	Grand means for hybrids
Bounty, 4101	78±1.904	80±2.432	92±3.815	92±2.399	88±2.782	83±2.609	81±1.886	79±1.893	120±3.614	91±2.604	90±0.915
[(D×J)×D] S_6, 4102		69±2.859	86±3.235	83±1.978	91±2.156	75±2.807	74±1.542	78±2.744	115±2.378	84±1.718	85± .796
[(J×BB)×BB] S_5, 4105			73±2.899	92±2.680	102±2.481	87±2.128	84±3.224	85±3.101	112±2.295	99±3.067	93± .979
[(D×RC)×D] S_3, 4103				81±3.077	95±1.103	78±3.021	81±2.712	82±2.334	121±3.259	99±2.335	91± .832
[(D×RC)×D] S_5, 4104					90±1.721	90±3.609	91±3.163	90±1.893	125±3.195	95±2.376	96± .876
Danmark, 4106						76±2.785	73±2.812	75±3.273	120±3.133	91±2.729	86± .977
[(D×RC)×D] S_6, 4107							71±2.151	73±2.538	115±2.770	91±2.698	85± .882
[(D×RC)×D] S_6, 4108								73±2.663	116±2.983	92±1.365	86± .844
(J×RC) F_9, 4109									100±2.795	122±1.472	118± .952
(J×RC) F_8, 4110										78±2.539	96± .778
Grand means for inbred lines and hybrids, respectively										79±0.815	93±0.280

* Symbols: D, Danmark; J, Johannisfeuer; RC, Red Currant; BB, Bonny Best.

TABLE 7

MEANS FOR NUMBER OF LOCULES PER FRUIT*

Variety or inbred line	Bounty 4101	[(D×J)×D] S_6 4102	[(J×BB)×BB] S_5 4105	[(D×RC)×D] S_5 4103	[(D×RC)×D] S_5 4104	Danmark 4106	[(D×RC)×D] S_6 4107	[(D×RC)×D] S_6 4108	(J×RC) F_9 4109	(J×RC) F_8 4110	Grand means for hybrids
Bounty, 4101	11.7±0.499	10.4±0.372	8.6±0.302	9.2±0.329	8.8±0.280	9.7±0.338	10.2±0.417	8.9±0.268	4.7±0.121	7.3±0.249	8.6±0.103
[(D×J)×D] S_6, 4102		16.1±0.438	9.0± .215	8.4± .204	8.0± .306	9.5± .256	9.4± .241	9.3± .370	9.6± .200	13.2± .306	9.6± .094
[(J×BB)×BB] S_5, 4105			7.0±0.150	7.3± .154	6.0± .161	7.8± .276	8.4± .338	7.5± .360	3.9± .090	6.4± .187	7.2± .082
[(D×RC)×D] S_5, 4103				7.0±0.270	5.9± .154	8.1± .468	8.0± .158	7.8± .243	3.7± .085	6.1± .175	7.2± .082
[(D×RC)×D] S_5, 4104					5.8±0.203	7.2± .176	6.3± .150	6.3± .132	3.8± .116	5.2± .249	6.4± .067
Danmark, 4106						8.0±0.335	8.7± .357	8.0± .233	4.7± .251	6.7± .355	7.8± .104
[(D×RC)×D] S_6, 4107							9.5±0.404	8.4± .299	4.5± .162	6.9± .192	7.9± .091
[(D×RC)×D] S_6, 4108								8.4±0.145	4.3± .141	6.3± .189	7.4± .087
(J×RC) F_9, 4109									3.9±0.058	6.0± .226	5.0± .055
(J×RC) F_8, 4110										9.6± .328	7.1± .081
Grand means for number of locules per fruit in inbred lines and hybrids, respectively										8.7±0.099	7.4±0.027

TABLE 8

MEANS OF WEIGHT (IN GRAMS) PER LOCULE*

Variety or inbred line	Bounty 4101	[(D×J)×D] S_6 4102	[(J×BB)×BB] S_5 4105	[(D×RC)×D] S_5 4103	[(D×RC)×D] S_5 4104	Danmark 4106	[(D×RC)×D] S_6 4107	[(D×RC)×D] S_6 4108	(J×RC) F_9 4109	(J×RC) F_8 4110	Grand means for hybrids
Bounty, 4101	10.6±0.751	10.6±0.514	14.2±1.182	10.1±0.704	10.5±1.084	10.4±0.640	11.1±1.099	12.6±0.522	6.5±0.239	5.9±0.369	10.2±0.259
[(D×J)×D] S_6, 4102		9.2±0.883	12.3±0.697	9.8± .361	10.1± .350	9.5±1.606	10.3± .520	10.2± .741	4.2± .162	4.2± .227	9.0± .235
[(J×BB)×BB] S_5, 4105			17.4±1.219	10.0± .411	13.2± .503	14.3±0.744	11.7± .941	14.2± .947	7.8± .421	6.7± .399	11.6± .248
[(D×RC)×D] S_5, 4103				8.0±0.488	9.2± .526	9.3±0.499	8.7± .482	9.2± .695	5.8± .287	4.4± .228	8.5± .164
[(D×RC)×D] S_5, 4104					9.5±0.562	10.0±0.619	9.9± .349	11.0± .388	5.2± .361	4.9± .305	9.3± .183
Danmark, 4106						11.8±0.929	10.4± .748	11.5± .553	5.8± .420	5.0± .311	9.6± .256
[(D×RC)×D] S_6, 4107							9.4±0.541	9.8± .592	6.0± .242	4.9± .228	9.2± .215
[(D×RC)×D] S_6, 4108								9.5±0.440	6.0± .277	5.4± .251	10.0± .197
(J×RC) F_9, 4109									3.0±0.139	2.7± .125	5.6± .099
(J×RC) F_8, 4110										1.8± .088	4.9± .094
Grand means for inbred lines and hybrids, respectively										9.0±0.218	8.8±0.064

* Symbols: D, Danmark; J, Johannisfeuer; RC, Red Currant; BB, Bonny Best.

The data furnish some information as to whether the inbred lines with superior combining ability can be selected on the basis of the number of ripe fruit per plant of the inbred lines. On the basis of number of ripe fruit per plant, lines 4109, 4110, 4103, and 4104 would have been selected as outstanding. Table 2 shows that these inbred lines also produced the greatest number of ripe fruit per plant in hybrid combinations. This is particularly true of the intraclass hybrids.

Weight of individual fruit

The data on weight of individual fruit are given in table 3. The figures show that the grand mean for the inbred lines is greater than the grand mean for the F_1 hybrids and also that the grand means of the hybrids of the inbred lines of classes 1 and 3 are less than the means of their respective inbred lines. The reverse is true for the same comparisons involving classes 2 and 4. Since classes 1 and 3 are composed of the larger-fruited inbred lines, these facts indicate at least partial dominance of smaller fruit.

Examination of the data in more detail reveals that the intraclass hybrids of class 1 show nonbeneficial heterosis; those of classes 3 and 4 show partial dominance of larger fruit; and the two inbred lines of class 2 do not differ sufficiently to permit conclusions. The inbred lines of class 1 do not all behave the same as regards interclass hybrids. The interclass hybrids of 4102 with the inbred lines of classes 2, 3, and 4 all show partial dominance of smaller fruit; those of 4101 and 4105 with the inbred lines of classes 2 and 3 show no dominance, or at best slight partial dominance of larger fruit. These latter inbred lines of class 1 when crossed with those of class 4 show marked partial dominance of smaller fruit. Another important fact can be deduced from the data for the interclass hybrids derived by crossing the inbred lines of class 1 with those of classes 3 and 4; namely, the fruit of the hybrids resulting from crossing inbred lines 4101 and 4105 of class 1 with those of class 3 are larger on an average than those resulting from crossing inbred 4102 with these same inbred lines of class 3. The reverse is true for the same comparisons involving the interclass hybrids of classes 1 and 4. This interaction is well established statistically. The interclass hybrids involving the inbred lines of classes 2 and 3 show no dominance, or at most a slight partial dominance, of smaller fruit. The interclass hybrids between inbred lines of classes 2 and 4 and those between inbred lines of classes 3 and 4 show marked partial dominance of smaller fruit. From these data it can be seen that the F_1 hybrids vary from no dominance, or at most slight partial dominance, of larger fruit, to slight partial dominance of smaller fruit, to marked partial dominance of smaller fruit, to nonbeneficial heterosis. This variation is great enough to have considerable influence on yield, which is significant when it is realized that weight of individual fruit multiplied by number of fruit gives yield.

As would be expected, the hybrids with the larger fruit are derived from those inbred lines having larger fruit.

Means for number of days from seeding to first fruit ripe

The means for number of days from seeding (April 17) to first fruit ripe are given in table 4. The grand mean for the inbred lines is somewhat greater than the grand mean for the F_1 hybrids, indicating heterosis for earliness of maturity, or at least complete dominance for this character in a majority of the hybrids. This is also indicated by the grand

Grand means for inbred lines and hybrids, respectively..... 9.0±0.218 | 8.8±0.064

*Symbols: D, Danmark; J, Johannisfeuer; RC, Red Currant; BB, Bonny Best.

means of the hybrids compared with the means of the inbred lines, as in every case but one the mean of the respective inbred line is greater than that of the grand mean of the hybrids. For the inbred line 4110 the reverse is true. However, the difference between the grand mean of the hybrids and the mean of the inbred line 4110 is not statistically significant. The intraclass hybrids of classes 1, 2, and 4 exhibit heterosis for earliness of maturity, whereas those of 3 do not. With the possible exception of 4102 crossed with the inbred lines of class 2, the interclass hybrids involving classes 1, 2, and 3 exhibit complete dominance for earliness. The two hybrid exceptions show heterosis. On the whole, the interclass hybrids involving 4109 tend to show a slight degree of heterosis for earliness, whereas those involving 4110 tend to show a degree of partial dominance that approaches complete dominance for earliness of maturity. These considerations show that again the character in the F_1 hybrids varies, ranging from partial dominance for earliness in some to heterosis in others. The F_1 hybrids 4101 × 4105 and 4109 × 4110 were 6 days earlier than their parents.

The difference between the earliest and the latest maturing variate is 24 days. The fact that the later inbred lines are earlier than such commonly grown varieties as Bonny Best and Earliana gives added significance to this statement. Also, in the majority of the cases earliness of maturity is completely dominant or shows heterosis. In general, the earlier hybrids resulted from crosses involving the earlier inbred lines.

Means for number of days from seeding to first bloom

The conclusions concerning dominance and heterosis that can be drawn from the data for number of days from seeding to first bloom are almost identical with those for number of days from seeding to first fruit ripe. As will be shown later, this does not necessarily mean that the variation in number of days from seeding to first fruit ripe is predominantly or largely controlled by number of days from seeding to first bloom.

Number of days from first bloom to first fruit set

Number of days from first bloom to first fruit set is not nearly so important in determining earliness and yield under the environmental conditions encountered at Cheyenne, Wyoming, as it is under the environments of some other areas. The grand means for the hybrids compared with the grand mean and means of the inbred lines show that on an average the hybrids exhibit partial dominance, complete dominance, or heterosis for the shorter period from bloom to first fruit set. Again, comparisons between the hybrids involving 4109 and those involving 4110 were interesting. In every case but one the number of days from first bloom to first fruit set was less for those hybrids having 4110 as a parent than the same period for either of the respective inbred parents. As regards the hybrids having 4109 as one of the parents, again in every case but one the values of the F_1 hybrids fell between those of the respective inbred parents or showed heterosis. In both instances the exceptions involved the hybrid 4110 × 4109. The value for this hybrid was not materially different from that of the inbred parent 4109, indicating complete dominance of the longer period. These data show that differences in number of days from first bloom to first fruit set do occur among the inbred lines and that these lines differ also in their expressions of

dominance and heterosis. The range in expression is from complete dominance of the longer period from bloom to first fruit set to heterosis of the shorter period from bloom to first fruit set.

Again it was found that the inbred lines having superior combining ability could be selected on the basis of their behavior.

Number of days from first fruit set to first fruit ripe

The data for mean number of days from first fruit set to first fruit ripe are given in table 5. The grand mean for inbred lines and the grand mean for hybrids are equal, showing that for this stage of development—on an average—the hybrids do not have advantage over the inbred lines. The grand means for hybrids compared with the means of the respective inbred lines indicate that as regards dominance and heterosis all hybrids do not behave alike; hence, a more detailed study of the data is essential.

A comparison of the interclass hybrids of classes 1 and 2 with those of 2 and 3 furnishes some information concerning combining ability. The data for the inbreds of classes 1 and 3 and for the interclass hybrids of these inbred lines crossed with 4103 of class 2 are given below:

Inbreds of class		F_1 hybrids involving 4103 and inbred lines of class	
1	3	1	3
Days	Days	Days	Days
54±0.471	53±0.433	49±0.327	50±0.563
51± .512	51± .348	48± .394	49± .314
51±0.277	51±0.427	47±0.340	50±0.327

The number of days from first fruit set to first fruit ripe was 46 ± 0.300 for the inbred line 4103. It can be seen from the tabulated data that, even though the means for the inbreds of classes 1 and 3 are identical within reasonable limits of sampling error, the differences between the comparable means of the F_1 hybrids listed under classes 1 and 3 cannot be accounted for by the deviations expected among random samples. These facts, in terms of expression of dominance, may be stated as follows. The hybrids resulting from crossing 4103 with the inbred lines of class 1 show partial dominance for the shorter period from first fruit set to first fruit ripe, whereas the hybrids from crossing this same inbred line with the inbred lines of class 3 show partial dominance for the longer period from first fruit set to first fruit ripe. Here is a clear case of difference in combining ability which should be examined in the light of the researches of Harland (**7, 8, 9, 10**). He found a mutation "crinkled dwarf" in *Gossypium barbadense* which was recessive to the normal condition. Normal in the F_1 hybrids between some strains of *G. barbadense* and certain strains of *G. hirsutum* showed incomplete dominance over crinkled, and in the F_2 generation a whole series of types appeared, in some of which normal was nearly recessive, in others intermediate, and in still others completely dominant. Hence, the dominance relations of normal and crinkled are controlled by a system of modifying genes. Also, Harland found that the dominance of normal or wild type over crinkled is due to the existence of a variety of normal alleles. Some strains carry normal alleles which in a certain genetic system of modifiers are nearly completely dominant over crinkled, whereas other normal alleles—presumably in the same genetic system of modifiers—are partially recessive to crinkled. It is clear that the inferior combining ability of the

inbred lines of class 3 as compared with those of class 1 can be due to differences in genetic systems of modifiers, differences in alleles, or both. Hence, this case of difference between inbred lines in combining ability is readily explainable by the genetic information available concerning dominance and dominance modifiers (**6, 20**).

Similar results to those shown by the tabulated data of the preceding paragraph were obtained with comparable hybrids between inbred line 4104 and the inbred lines of classes 1 and 3. Also, from table 5 it can be seen that the interclass hybrids between the inbred lines of classes 2 and 3 show partial dominance for the longer period, whereas the interclass hybrids between the inbred lines of classes 3 and 4 show it for the shorter period. A difference in degree of partial dominance for the shorter period is shown by comparing the values of the interclass hybrids involving inbred lines of classes 3 and 4 with the values for the hybrids involving inbred lines of classes 1 and 4. Both groups of hybrids exhibit partial dominance for the shorter period, but this developmental stage is longer in the latter. The values for the intraclass hybrids of classes 1 and 2 indicate that complete dominance, or even heterosis, may occur for fewer days from first fruit set to first fruit ripe.

In summary, it can be said that the expression of dominance and heterosis ranges from partial dominance of the greater number of days from first fruit set to first fruit ripe to complete dominance—or perhaps even a slight degree of heterosis—for fewer days from first fruit set to first fruit ripe. The hybrids with fewer days from first fruit set to first fruit ripe were derived from the inbred lines having this same characteristic.

Spread of plant

The data on spread of plant are given in table 6. The grand means show that in general the F_1 hybrids exhibited heterosis for increased spread of plant. The hybrids within class 3 did not exhibit heterosis, whereas the intraclass hybrids of all the other classes did. With the possible exception of 4103 × 4106, the interclass hybrids between members of classes 2 and 3 exhibited complete dominance for greater spread of plant. An interesting comparison may be made between the grand mean of the hybrids involving inbred lines 4104 and the grand mean of those involving 4110 as compared with the means of these two inbred lines. The mean spread of plant of 4104 is considerably greater than that of 4110, but the two grand means for the hybrids are equal.

The expression of spread of plant in the F_1 hybrids ranges from that of partial dominance for greater spread to a rather marked degree of heterosis. In general, the inbred lines having the greatest spread of plant produce those F_1 hybrids superior in this respect.

Height of plant

The conclusions drawn from the data for height of plant are so similar to those for spread of plant as not to warrant special discussion.

Number of locules per fruit

The data on number of locules per fruit are given in table 7. The grand mean for the inbred lines is seen to be greater than that for the hybrids, indicating partial dominance for fewer locules per fruit. That such probably is the case is also borne out by the grand means of the hybrids as compared with the means of their respective inbred lines.

This conclusion is found to hold for all intraclass hybrids, with the possible exception of 4101 × 4102, and for all interclass hybrids involving classes 1, 2, and 3. The following deductions can be made regarding the interclass hybrids involving inbred line 4109. Those hybrids resulting from crossing with the inbred lines of class 1 range from complete dominance of fewer locules per fruit to no dominance; those resulting from crossing with inbred lines of class 2 show nonbeneficial heterosis; and those resulting from crossing with inbred lines of class 3 show partial dominance of fewer number of locules per fruit. In the interclass hybrids involving 4110, it can be seen that —with the exception of the hybrid 4110 × 4102—all show nonbeneficial heterosis. The hybrid 4110 × 4102 shows no dominance. The hybrids involving 4102 also exhibit a nonuniform behavior pattern. The hybrid 4101 × 4102 shows slight nonbeneficial heterosis or no dominance; the hybrids resulting from crossing with inbred lines of classes 2 and 3 show partial dominance of fewer locules; and the hybrids resulting from crossing with inbred lines of class 4 show no dominance. The expression of number of locules per fruit varies from no dominance to nonbeneficial heterosis of fewer locules per fruit.

Weight per locule

The data on weight per locule are given in table 8. Probably one of the most striking features of the data is the range in expression of the character. The weight per locule varies from 1.8 gm. for inbred line 4110 to 17.4 gm. for line 4105. The interclass hybrids involving the inbred lines 4109 and 4110 (with the exception of 4103 × 4109) showed partial dominance of smaller weight per locule. The hybrids resulting from crossing inbred line 4101 with inbred lines 4107 and 4108, and those resulting from crossing inbred line 4102 with inbred lines 4107 and 4108, exhibited a slight degree of heterosis. The differences in magnitude of the means of these inbred lines were slight. Where the differences between the means of the inbred lines were marked, the resulting hybrid or hybrids generally exhibited partial dominance of less weight of locule per fruit.

The range in expression of weight per locule in the F_1 hybrids was from partial dominance of less weight per locule to heterosis of greater weight per locule. The F_1 hybrids with the greater weight per locule were derived from those inbred lines having greater weight per locule.

Relation between yield, number of ripe fruit per plant, and size of fruit

The future trend in methods of breeding tomatoes will be determined largely by the relation between yield, number of ripe fruit, and size of fruit. If the genetic relations and the manner of inheritance of the genes differentiating the characters are such that those giving the desired results can be easily and quickly recombined into individual inbred lines, then F_1 hybrids will have little if any importance in the commercial production of tomatoes, because it will be easier to grow the crop from inbred lines in which the desirable characters have been attained. On the other hand, if the genetic relations and the manner of inheritance of the genes differentiating the characters are such that they cannot be easily and quickly recombined into individual inbred lines, then the use of F_1 hybrids may become of increasing importance.

The relation between yield and the

two characters—number and size of fruit—is clearly defined, as yield is the product resulting from multiplying the value of one of these latter two characters by that of the other. When such a numerical system prevails, the expression of dominance and heterosis for the two independent characters gains added importance in the production of tomatoes from F_1 hybrids, because moderate increases in either number or size of fruit result in substantial increases in the dependent character yield. Likewise, moderate improvement of these two characters in the inbred lines may be expected to result in substantial increases in the yielding ability of the inbred lines themselves. An understanding of the numerical system involved emphasizes the importance of working with the component characters of yield, thereby permitting a more reliable evaluation of the gains made in either component. In turn, this makes the breeding program more flexible by allowing judicial shifts in the emphasis placed upon either of the components; hence the entire program is facilitated.

Probably one of the most important problems is the determination of the possibilities for the continued improvement of inbred lines and hybrids by increasing number of fruit and size of fruit. It is apparent that the considerable number (183) of ripe fruit per plant of the hybrid 4110 × 4109 cannot be recombined with the larger size (94 gm.) of fruit possessed by Danmark, owing to the physiological and morphological limitations of these plants. However, it is desirable to know whether the limit of recombination of greater number of fruit and larger size has been reached in the inbred lines and hybrids. To be more specific, can the number of ripe fruit the size of those of Danmark be materially increased? Data bearing on this question are tabulated below:

Character	Danmark	F_1, 4110×4109	Theoretical F_1 hybrid or inbred line
Yield (gm.)	828.0± 108.0	2876.0± 177.0	2876.0
Size (gm.)	94.0± 6.39	16.0± 0.68	94.0
Number	8.8± 1.08	183.2± 13.32	30.6

The actual yields, weights of individual fruit, and numbers of fruit are given for Danmark and the F_1 hybrid 4110 × 4109, whereas the values for the theoretical F_1 hybrid or inbred line are based on the yield of the F_1 hybrid 4110 × 4109 and the fruit size of Danmark. From a consideration of this tabulation it seems that F_1 hybrids or inbred lines can be obtained which will combine the fruit size of Danmark with a considerably increased number of ripe fruit. Such F_1 hybrids or inbred lines would be superior to Danmark in yielding ability.

However, unfavorable linkage, pleiotropy, number of gene pairs differentiating the contrasted characters, or a combination of the three may make such an accomplishment exceedingly difficult or practically impossible. Information bearing on whether such is the case can be obtained by considering the advances already made. Some of the more pertinent data are tabulated below:

Character	F_1, 4110×4102	F_1, 4101×4103
Yield (gm.)	2428.0± 150.0	1827.0± 196.0
Size (gm.)	55.0± 2.93	89.0± 3.66
Number	44.5± 2.52	20.5± 2.27

These values should be compared with those of Danmark and of the F_1 hybrid 4110 × 4109 (given in the preceding tabulation). The number of fruit that ripened in hybrid 4110 × 4102 is approximately five times that of Danmark,

and the weight of individual fruit is approximately four times that of the F_1 hybrid 4110 × 4109. Also, the size of fruit of the F_1 hybrid 4101 × 4103 is not significantly different from that of Danmark, whereas the number of fruit that ripened is more than twice that of Danmark. Although decided advances have been made in combining increased number and size of fruit in inbred lines, they are not nearly so impressive as those made in combining these two desirable characters in the F_1 hybrids.

Probably equally pertinent evidence bearing on the problem as to how much progress can be made in combining more and larger fruits is furnished by a consideration of the advances that have been made in breeding superior inbred lines. Inbred lines 4108 and 4110 were crossed in an attempt to increase size of fruit and increase or at least maintain earliness. From the F_2 and first generation backcrosses, a number of individuals were selected which fulfilled these requirements. Also, crosses were made between Danmark and Ponderosa in an endeavor to obtain inbred lines that matured as early as Danmark and possessed considerably larger fruits. From some of the backcross progenies which had been inbred by self-pollination for four generations after the original backcross, strains were selected in 1942 which were as early maturing as Danmark and which had fruits one and one-half times as large. F_1 hybrids involving some of these improved strains should have fruits of acceptable commercial size and in addition should produce considerably greater yields than any of the commercial varieties available at the present time.

That the numerical system clearly defines the relation between yield and the two characters number of ripe fruit and size of fruit has been shown. Since number of ripe fruit multiplied by weight of individual fruit gives yield, all the variability (variance) of yield must be accounted for by the multiple regression of yield on number of ripe fruit and size of fruit. The relative influence of these two characters on yield has not been determined; that is, what percentage of the variation attributable to yield has been accounted for by number of ripe fruit and what percentage by weight of individual fruit. Again, regression would be expected to yield some information.

Before going into an interpretation of the data, some facts should be mentioned. It will be remembered that the design of the experiment was that of a randomized complete block, composed of ten blocks with fifty-five variates (hybrids and inbred lines) per block; hence the sources of variation are as shown in table 9. The variation between means of hybrids and inbred lines is largely genetic; that due to blocks is mainly environmental; and that due to interaction may be both. Also, in the original cross from which seven of the ten inbred lines resulted, numerous fruit and small size were combined in one parent and fewer fruit and larger size in the other. Even though much progress has been made toward recombining these two desirable characters, comparatively speaking greater number of ripe fruit and smaller size still tend to be associated. Then, even though the maximum limit of recombination of greater number of fruit and larger size has not been attained in the hybrids and inbred lines, a negative relation might be expected between these two characters.

The percentages of the variances of weight of all fruit that ripened per plant (yield), number of fruit that ripened per plant, and weight of an individual fruit (size) that can be accounted for by re-

gression are given in table 9. When the association between the characters is negative, the respective values are followed by a negative sign in parentheses. All values were transformed to logarithms for calculating regressions. Considering the variation attributable to differences between hybrids and inbreds, the table shows that 89.82% of the variance of number of fruit and size of fruit are accounted for by the regression of these two characters upon each other. The relation is negative. Eighty-four and ninety-five hundreths per cent of the variance of yield is accounted for by the regression of yield on number of fruit and 56.20% by yield on size. In the first case the association is positive and in the latter negative. The multiple regression of yield on the other two characters accounted for practically all (99.98%) the variability of yield. This shows that for all practical purposes the correct transformation of the data was used. The fact that the association between size of fruit and yield is negative, whereas the association between number of ripe fruit and yield is positive, indicates that in this material number of ripe fruit has a greater influence in bringing about an increase in yield than size of fruit. The negative association between number of ripe fruit and size of fruit seems to be due to the way these two characters were combined in the original parents and inbred lines, rather than to any other genetic or physiological phenomena. From the data listed under blocks it is evident that all the relations are positive and that number of ripe fruit per plant accounts for a considerably greater proportion of the variance of yield than does size of fruit. Also, the number of ripe fruit per plant and the size of fruit are practically independent of each other. Regression accounted for very little of the variance of either character. From these data, and from those given under interaction and total of table 9, the following conclusions seem logical. Indications are that number of ripe fruit per plant accounted for approximately 85% of the genetic variability due to differences in yield and about 95% of the environmental variability due to differences in this character; whereas size of fruit accounted for the remaining 15% and 5%, respectively.

The following summary concerning

TABLE 9

PERCENTAGE OF VARIANCES OF WEIGHT OF RIPE FRUIT PER PLANT (YIELD), OF NUMBER OF FRUIT THAT RIPENED PER PLANT (NUMBER), AND OF WEIGHT OF AN INDIVIDUAL FRUIT (SIZE) THAT ARE ACCOUNTED FOR BY REGRESSION

Independent characters	Dependent characters		
	Simple regression		Multiple regression
	Size (%)	Yield (%)	Yield (%)
Main effects			
Hybrids and inbreds			
Number	89.82 (−)	84.95	99.98
Size		56.20 (−)	
Blocks			
Number	0.38	95.82	99.99
Size		6.97	
Interaction			
Hybrids and inbreds × blocks			
Number	0.26 (−)	71.09	99.97
Size		24.35	
Total			
Number	67.93 (−)	71.21	99.99
Size		15.35 (−)	

the interrelation of yield per plant, number of ripe fruit, and size of fruit seems justified: It should be possible materially to increase yields of commercially acceptable tomatoes by recombining greater number of fruit that ripen with larger size of fruit. In fact, inbred lines are now available at the Cheyenne Horticultural Field Station which should make this possible if F_1 hybrids are grown for production of the commercial crop.

Relation between number of ripe fruit, days from seeding to first fruit ripe, spread of plant, and height of plant

The method of regression was employed also to study the relation between number of ripe fruit per plant, number of days from seeding to first fruit ripe, spread of plant, and height of plant. The variation analyzed was that due to differences between hybrids and inbred lines. Fifty-six per cent of the variance of number of ripe fruit per plant was accounted for by the regression of this character on spread of plant; 63% of the variance of number of days from seeding to fruit ripe was accounted for by the regression of this character on spread of plant; and 93% of the variance of number of ripe fruit per plant was accounted for by the regression of this character on number of days from seeding to fruit ripe. As would be expected in the latter two instances, the association was negative. Apparently, spread of plant influences number of ripe fruit. Also, earliness of maturity would be expected to have a decided influence on number of fruit that ripen in locations such as Cheyenne, Wyoming, where freezes and frosts occur early. The results for height of plant were very similar to those for spread of plant and hence need not be considered further. The percentage of the variance of number of ripe fruit accounted for by multiple regression is very little greater than that accounted for by the regression of this character on earliness of maturity. Then it would seem that size of plant as determined by measurement of spread of plant and height of plant may be having an effect on number of ripe fruit per plant by increasing earliness of maturity.

If these conclusions are correct, one might expect to find some hybrids and inbred lines that combine greater number of ripe fruit per plant, earliness of maturity, and greater plant spread. That these three desirable characters do occur in some F_1 hybrids and inbred lines is shown by the data in columns 4109 and 4110 of tables 2, 4, and 6. The possibilities for increasing yield by combining these three characters are interesting. The fact that inbred line 4110 produced 1868 gm. of ripe fruit with a plant spread of only 78 cm. indicates that it should be possible to attain considerably higher yields by combining the plant spread (100 cm.) of 4109 with the desirable characters of 4110. The F_1 hybrid 4110 × 4109 as compared with the inbred parents represents an increase in number of ripe fruit per plant, earliness of maturity, and plant spread which is accompanied by a decided increase in yield.

The relation between height of plant and the other characters is very similar to that for spread of plant and the other characters and therefore does not need discussion.

The relation between size of fruit and number of days from date seeded to first fruit ripe should now be considered. In 1941, studies were conducted to determine what progress had been made in recombining extreme earliness and large size of fruit. The data are summarized in table 10. They show that size of fruit as determined by weight can be increased

five to thirteen times that of the earlier parent, Red Currant, without loss of earliness. In fact, selection 13–7, which is in the F_7 generation, is somewhat earlier than Red Currant and has fruits thirteen times as large. From tables 3 and 4 it can be seen that some crosses with this same inbred line 13–7 (4109) have fruits two to three times as large as inbred line 13–7 (4109) and are just as early—or earlier—maturing. These data indicate that large size of fruit and extreme earliness of maturity can be recombined, either in F_1 hybrids or inbred lines.

TABLE 10

MEAN NUMBER OF DAYS FROM SEEDING TO FIRST FRUIT RIPE (MATURITY) AND MEAN WEIGHT (SIZE) OF INDIVIDUAL FRUIT

Strain or hybrid*	Maturity (days)	Size (gm.)
Red Currant	128.3±1.183	1.18±0.057
J×RC, F_1	126.0±0.954	7.1 ±0.233
J×RC, F_7, 13–7	124.5±0.582	16.0 ±0.447
J×RC, F_7, 196–6	126.3±0.932	15.0 ±0.471
J×RC, F_7, 379–5	128.7±0.895	14.2 ±0.533
Johannisfeuer	141.9±1.130	51.1 ±1.841
D×J, F_1	140.2±1.209	58.2 ±1.788
D×RC, F_1	124.5±0.991	8.6 ±0.163
Danmark	148.1±0.883	67.6 ±1.851

* Symbols: RC, Red Currant; J, Johannisfeuer; D, Danmark.

RELATION BETWEEN DAYS FROM SEEDING TO FIRST FRUIT RIPE, DAYS FROM SEEDING TO FIRST BLOOM, DAYS FROM FIRST BLOOM TO FIRST FRUIT SET, AND DAYS FROM FIRST FRUIT SET TO FIRST FRUIT RIPE

Days from seeding to first fruit ripe logically may be partitioned into days from seeding to first bloom, days from first bloom to first fruit set, and days from first fruit set to first fruit ripe (16). Obviously, the numerical system is such that the values of the different characters are additive. Hence, if these characters can be recombined in various ways in inbred lines, and through these inbred lines in F_1 hybrids, then it can be concluded that the end effects of some of the genes differentiating earliness of maturity (days from seeding to first fruit ripe) are additive. The following facts can be derived from tables 4 and 5 and the following data. Inbred lines 4109 and 4110 were derived from the same cross—Johannisfeuer × Red Currant. The means for number of days from date seeded to first fruit ripe are the same, 124 days, for both inbred lines. Likewise, the number of days from date seeded to first bloom are identical. But, comparatively speaking, the period from first bloom to first fruit set is long (9.2±0.586 days) for inbred line 4109 and short (6.4±0.514 days) for 4110. The period from first fruit set to first fruit ripe is shorter in 4109 than in 4110 (table 5). This one set of comparisons alone shows that these three characters can be recombined in different combinations. Also, the same conclusion can be drawn from a comparison of inbred lines 4103 and 4106. All three of the short periods of the three independent characters are combined in the F_1 hybrid, 4104 × 4109.

Again the method of regression should add to our understanding of the relation between the characters. The data were transformed to logarithms before the calculations were made. The percentages of the variances that are accounted for by regression are given in table 11. Only the analysis of the variation due to differences between hybrids and inbreds is given. The data in table 11 support the following conclusions: The majority of the variation of the independent characters is independent; that is, not attributable to covariation. This is in accord with the previous data showing that

the desirable characters can be recombined in inbred lines and F_1 hybrid populations. Also, it seems that number of days from first fruit set to first fruit ripe has a greater influence on number of days from seeding to first fruit ripe than do number of days from seeding to first bloom or number of days from first bloom to first fruit set. The fact that 99.8% of the variance was accounted for by the multiple regression of number of days from seeding to first fruit ripe on the independent characters shows that the transformation to logarithms placed the data on the proper scale of measurement.

TABLE 11

PERCENTAGE OF VARIANCE OF NUMBER OF DAYS FROM SEEDING TO FIRST FRUIT RIPE (MATURITY), NUMBER OF DAYS FROM SEEDING TO FIRST BLOOM (BLOOM), NUMBER OF DAYS FROM FIRST BLOOM TO FIRST FRUIT SET (SET), AND NUMBER OF DAYS FROM FIRST FRUIT SET TO FIRST FRUIT RIPE (RIPE) THAT ARE ACCOUNTED FOR BY REGRESSION. ONLY THE ANALYSIS OF VARIATION DUE TO DIFFERENCES BETWEEN HYBRIDS AND INBREDS IS GIVEN

Independent characters	Dependent characters		
	Set (%)	Ripe (%)	Maturity (%)
Bloom	18.44 (−)	24.43	56.24
Set	.....	0.56	0.14
Ripe	.....	.....	85.19
Multiple regression	.....	.....	99.80

RELATION BETWEEN WEIGHT OF INDIVIDUAL FRUIT, NUMBER OF LOCULES PER FRUIT, AND WEIGHT PER LOCULE

Weight of individual fruit may be partitioned into number of locules per fruit and weight per locule. Again, the numerical system is such that the effects of the two independent characters are multiplicative. Also, all the variation of weight of individual fruit must be accounted for by variation in number of locules per fruit and weight per locule. That greater number of locules and greater weight per locule can be combined is shown by inbred lines 4101 and 4102. Line 4101 has 11.7 ± 0.499 locules per fruit which weigh 10.6 ± 0.751 gm. per locule, and line 4102 has 16.1 ± 0.438 locules per fruit which weigh 9.2 ± 0.883 gm. per locule.

The method of regression was employed to obtain further information concerning the relation between the characters. Only 10% of the variation of number of locules and weight of locules was accounted for by covariation (table 12). Therefore, of the magnitudes and range of the characters accounted for in this material there would not seem to be any appreciable physiological or morphological limitations on the recombination of these two characters. In this material the values of covariation indicate that weight per locule is probably somewhat more influential in regulating size of fruit than is number of locules. The multiple regression of size of fruit on number of locules and weight per locule accounted for 99.76% of the variation of

TABLE 12

PERCENTAGE OF VARIANCES OF WEIGHT OF INDIVIDUAL FRUIT (FRUIT SIZE), NUMBER OF LOCULES PER FRUIT (NUMBER), AND WEIGHT PER LOCULE (LOCULE SIZE) THAT ARE ACCOUNTED FOR BY REGRESSION. ONLY THE ANALYSIS OF VARIATION DUE TO DIFFERENCES BETWEEN HYBRIDS AND INBREDS IS GIVEN

Independent characters	Dependent characters	
	Locule size (%)	Fruit size (%)
Number	10.09	52.71
Locule size	.....	77.64
Multiple regression	.....	99.76

size of fruit, showing that the regression is primarily logarithmic rather than linear.

Discussion

An important contribution of these studies is the information they furnish concerning the nature of combining ability. The partitioning, in so far as practical, of the main characters into their components has been particularly helpful in showing the relation between combining ability and genetic diversity, dominance, and heterosis. An extensive review of the literature concerning dominance and heterosis is not within the province of this paper. For such a review, and for some of the more pertinent facts, reference is made to GOLDSCHMIDT (6), WADDINGTON (20), WRIGHT (22), BEADLE and COONRADT (4), and POWERS (14, 15). Particularly pertinent to an understanding of the nature of combining ability is the work of HARLAND (7, 8, 9, 10) discussed earlier in this paper (p. 257). From this genetic information concerning dominance and heterosis, and since from the standpoint of physiological genetics dominance and heterosis have been shown (14, 15) to be different degrees of expression of the same phenomenon, it is clear that combining ability of inbred lines is dependent on the nature and diversity of the alleles differentiating the contrasted characters, the intraallelic and interallelic interactions of the genes, the system of modifying genes, and finally the interaction between the genotypic milieu and the environment. In brief, the combining ability of these inbred lines is dependent upon genetic diversity and the phenomenon of dominance and heterosis, and the available information concerning these genetic phenomena is sufficient to account for all the known facts concerning combining ability.

BARRONS (2) points out that commercial seed companies hesitate to enter the field of F_1 hybrid tomato-seed production owing to the difficulties and costs involved. Hence, any means of reducing this cost and these difficulties would materially facilitate the use of F_1 hybrids in commercial production. TERNOVSKI and MISSURA (18) have shown that male sterility can be induced in a certain percentage of the plants resulting from X-ray irradiation of seed. By X-raying seed of those plants found to be desirable for the production of F_1 hybrids and growing large populations, it seems that male sterile inbred lines could be established. These could then be used in producing the hybrid seed—either by insect or by hand pollination. In case insect pollination of the induced male steriles is found to be feasible, the cost and difficulties of producing hybrid seed would be greatly reduced. Even though insect pollination did not prove satisfactory, hand pollination would be much easier owing to the elimination of the necessity for emasculation. The induction of male sterility in inbred lines to be used in making F_1 hybrids is of sufficient promise to warrant intensive studies on the production of male steriles by irradiation, on determining the value of such inbreds in a tomato-breeding program, and on working out methods of utilizing them to obtain F_1 hybrid tomatoes for commercial production.

Summary and conclusions

1. The yield of ripe tomatoes in those areas having a short growing season can be greatly increased by utilizing F_1 hybrids for producing the crop. All the high producing F_1 hybrids have at least one parent derived from crosses between *L. esculentum* Mill. and *L. pimpinellifolium* (Jusl.) Mill. The most outstand-

ing F_1 hybrid yielded by weight three times as much ripe fruit per plant as did Danmark. This increased yield was due primarily to an increase in earliness. The range in expression of dominance and heterosis varied from no dominance to decided beneficial heterosis.

2. In respect to number of ripe fruit per plant, the range in expression of dominance and heterosis varied from partial dominance of fewer fruits per plant to heterosis for greater number of fruits per plant. This is significant when considering that the range in number of ripe fruit per plant varied from 2.9 to 183.

3. As regards weight of ripe fruit, the F_1 hybrids varied from no dominance—or at most, slight partial dominance—of larger fruit to slight partial dominance of smaller fruit to marked partial dominance of smaller fruit to nonbeneficial heterosis. This variation is sufficient to play considerable part in determining yield.

4. The difference between the earliest and the latest maturing variate is 24 days. That the latest maturing inbred lines and F_1 hybrids are earlier than such commonly grown varieties as Bonny Best and Earliana gives added significance to this fact. Also, in the majority of cases earliness of maturity is completely dominant or shows heterosis.

5. The range in expression of dominance and heterosis is from complete dominance of the longer period from bloom to first fruit set to heterosis of the shorter period from bloom to first fruit set.

6. For number of days from first fruit set to first fruit ripe the expression of dominance and heterosis ranges from partial dominance of the greater number of days from first fruit set to first fruit ripe to complete dominance or perhaps even a slight degree of heterosis for fewer days from first fruit set to first fruit ripe.

7. For spread of plant the expression of the character in the F_1 hybrids ranges from partial dominance for greater spread of plant to a rather marked degree of heterosis. The conclusions for height of plant are very similar to those for spread of plant.

8. The expression of number of locules per fruit in the F_1 hybrids varies from no dominance to nonbeneficial heterosis of fewer number of locules per fruit.

9. In the F_1 hybrids the expression of weight per locule varies from partial dominance of smaller weight per locule to heterosis of greater weight per locule.

10. The interrelation of yield per plant, number of ripe fruit, and size of fruit is such that it should be possible materially to increase yields of commercially acceptable tomatoes by recombining greater number of fruit that ripen with larger size. In fact, inbred lines are now available at the Cheyenne Horticultural Field Station which should make this possible if F_1 hybrids are grown for production of the commercial crop.

11. The relation between number of ripe fruit, days from seeding to first fruit ripe, and spread of plant is such that it should be possible to obtain inbred lines and F_1 hybrids having the desired combination. Extreme earliness and large size of fruit probably can be recombined.

12. Days from seeding to first fruit ripe logically may be partitioned into days from seeding to first bloom, days from first bloom to first fruit set, and days from first fruit set to first fruit ripe. The relation between these characters is such that the shorter periods of development can be combined to produce a decidedly earlier maturing tomato.

13. The relation between number of locules per fruit and weight per locule is such that the greater number of locules and the greater weight per locule can be recombined to give increase in size of fruit.

14. In general, the data show that the inbred lines having superior combining ability—as determined by the behavior of F_1 hybrids in respect to the eleven characters studied—are themselves superior in comparison with other inbred lines. However, the exceptions to this statement are of sufficient importance so that they cannot be ignored in a plant-breeding program.

15. From these studies dealing with the degree of expression of the phenomenon of dominance and heterosis for the eleven quantitative characters, and from the information obtained by partitioning some of the main characters into their components, it is apparent that the greatest strides in breeding can be made by utilizing F_1 hybrids rather than inbred lines for the production of commercial crops.

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LEAF AND BUD INITIATION IN THE GRAMINEAE

B. C. SHARMAN

Introduction

In spite of the widespread distribution and the economic importance of the order, there appears to have been very little investigation of the mode of initiation of the leaf and bud in the Gramineae. What little literature there is fails to give an exact picture of the earliest stages: it is hoped that the present study may elucidate some of the more salient features.

DOULIOT (12), studying mainly *Phragmites communis* Trin., stated that the new primordium appears as an annular swelling encircling the stem tip. He noticed that the epidermis and the underlying cells both play a part in the production of the first up-pushing but considered that after this stage the whole leaf is derived by division of the cells forming the edge of the ring. He considered that the ring is the future sheath and that the lamina grows at the expense of the cells of the free edge or border of the sheath, growing by a terminal cell.

BARANETZKY (5), in *Bambusa arundinacea* Willd., also noticed periclinal divisions in the epidermis at the initiation of the primordium, but he was not certain that it was a constant feature.

BUGNON (11), studying *Dactylis glomerata* L. and *Melica altissima* L., showed that the new primordium originates on one side of the apex and that the periclinal divisions in the epidermis and underlying layer spread sideways from the point of origin to give the small ringlike protuberance. He stated that the ring might be regarded as the lamina and not the sheath and that it possesses a great number of terminal initials all along its free edge. He was of the opinion that the cells underlying the epidermis themselves divide periclinally and so help to raise the original up-pushing and contribute to the tissue of the developing leaf.

ARTSCHWAGER (3) gave a drawing of a longitudinal section of the shoot apex of *Saccharum officinalis* L., but he was mainly concerned with the later development of the leaves and axis and made no mention of the mode of origin of the primordia.

RÖSLER (18) stated that in *Triticum vulgare* Host., and also in *Avena sativa* L. and *Secale cereale* L., the original protuberance (the very young primordium —*Blatthöcker*) arises solely from the dermatogen, although he did not seem completely convinced that the cells of the layer immediately inside do not contribute something during the later stages.

PORTERFIELD (17) examined the anatomy of the apex of *Phyllostachys nigra* Munro from the point of view of HANSTEIN'S histogens. He does not seem to have been very certain of the way the leaves are initiated, but he was clearer than most of his predecessors about the shoot as a whole, and he also noticed the peculiar orientation of the axial cells foreshadowing the bud.

KLIEM (15) concluded that in *Avena sativa* some of the inner tissue of the leaf base is probably derived from the "corpus."

ABBE, RANDOLPH, and EINSET (1) studied the shoot apex of *Zea mays* L. in relation to the size of the leaves during a small part of the life history of the plant. They were not, however, directly concerned with the architecture of the apex or primordia.

The writer (22) regarded the leaf of *Zea mays* as derived from both the dermatogen and the cells immediately inside this layer. It was also pointed out that in the young primordium there is no distinction between sheath and lamina, and that the difference arises later, possibly owing to the different conditions under which each part is developing. Since then, in an attempt to establish the true course of events in the early stages, a study has been made of the origin of the primordia in a wide range of cereals and herbage grasses. In all the cases examined the main features were found to be the same, so that the following account which is presented for *Agropyron repens* is regarded as being applicable to any other member of the order.

Material and methods

Couch grass, *Agropyron repens* Beauv., was chosen because it has a comparatively long apex bearing a number of leaves in various stages of early development. At the same time, *Agropyron* offered the possibility of comparing the apex of an aerial shoot with that of a subterranean rhizome. Its close relationship to *Triticum* also influenced the choice.

Numerous vacuome and cytoplasmic fixatives were tried, but modifications of Chamberlain's formalin-fatty-acid-alcohol mixture gave the best results. Although it was never possible to obtain material completely without retraction of the outer wall of the dermatogen cells, the following mixture was about the best:

Propionic acid	6.5 cc.
Formalin (40% aqueous solution)	10
Ethyl alcohol (70%)	up to 100

One difficulty in the fixation of grass apices is caused by differences in the maturity of the different shoots. Although a mixture may be good for the apex of a comparatively mature shoot, it will often cause considerable shrinking of the dermatogen cells of the apex of a newly emerged shoot. Before being fixed, the shoots had the outer leaves removed, down to and including the one with its lamina just fully exposed as far as the ligule. This ensured that the material was free from dense, heavily lignified tissue and would cut easily. It also proved useful when examining the sections, since it allowed a quick assessment of the approximate maturity of the outer few leaves.

Dehydration was carried out in absolute alcohol kept over anhydrous copper sulphate, in the manner suggested by BALL (4). Infiltration was accomplished via chloroform, since this seemed to render the tissues less hard and brittle than xylol. The absolute alcohol-chloroform mixtures were also kept over anhydrous copper sulphate.

Sections were usually cut at 4–6 μ, with the object of cutting most cells at least twice. In order to stain the cell walls of the meristematic tissues, the sections were stained—as described in more detail elsewhere (23)—in dilute aqueous safranin (1:25,000) after mordanting in a 2% aqueous solution of zinc chloride, followed by a bath consisting of 5 gm. tannic acid, 2 gm. orange G, 4 drops concentrated hydrochloric acid, and water to 100 cc. The sections were then carried through 5% tannic acid solution, followed by a 1% solution of iron-alum. As a result the cell walls were blue-black and the nuclei shades of yellow to orange, varying according to their metabolism. In actively dividing cells the cytoplasm was usually yellow or dark orange and in more quiescent cells pale yellow to grey. Procambial and vascular tissues showed good differentiation, while starch could easily be recognized, even in meristematic cells.

From time to time dissected apices were cleared in Eau de Javelle. It was found that their clarity could be greatly improved if they were taken carefully up to 50% alcohol, when they were useful for obtaining a general view of the form changes in the solid.

All drawings were made with the aid of a microprojector: in the case of longitudinal sections especially, it was usually necessary to combine two or more sections to keep the view in the appropriate plane.

Observations

Morphology of shoot

The underground rhizome produces only scale leaves, which are usually undivided into laminae and sheaths. When the soil surface is reached in spring, a succession of normal leaves is produced, beginning with a transitional leaf (usually only one) which bears a small lamina about 0.5 mm. long. Each successive leaf lamina is longer, until about the third from the inflorescence is reached—when the sizes decrease a little. Considering the transitional as the first, the main axis usually produces twelve leaves in all. There may perhaps be some slight variation in number from year to year, but during any one season about 80% of the main shoots conform to one number and the remaining 20% deviate by only one leaf more or one less.

The shoot apex of the rhizome growing horizontally underground is an elongated dome-shape, seen in figure 1*A* and in longitudinal section in figure 2. From this stage until the shoot bears two to three fully developed green leaves, the apex appears as in figure 1*B–C′*, when about three to five primordia may be observed in various stages of maturity. Each primordium appears as a small protuberance on one side of the apex a little distance back from the extreme tip (fig. 1*C*). This is quickly transformed into a crescent and then into a collar almost completely surrounding the apex. At first the growth appears to be mainly at right angles to the surface of the axis, but it soon takes on a more vertical trend. The first-formed part is always farthest ahead, and soon the primordium forms a cowl or hood growing over and inclosing the apex (fig. 1*A–D*). Growth from this stage on is extremely rapid; only about two plastochrones separate it from the time when the tip of the leaf is first beginning to appear from the center of the shoot, and only three or four until the lamina is fully exposed and elongated.

As the shoot becomes more mature the apex becomes more elongated and bears more primordia (fig. 1), until—when approximately the fifth or sixth green leaf is fully exposed—there are about eight between the youngest and the one just overtopping the apex (fig. 1*D*, D′).[1] Figure 3 shows a similar apex in longitudinal radial section, cut in the plane of the leaf insertions. Although at this stage the primordia look exactly like those which arose earlier, presumably only those at the base of the apex will develop into normal green leaves, since the total on a fully developed shoot is twelve. The rest will presumably cease development early, and at the most appear as small ridges subtending the spikelets in the inflorescence. The stage seen in figure 1*D* and *D′* is followed by

[1] It is not easy to find a natural scale for comparing one apex with another. Counting the number of successive primordia from the youngest to that just sufficiently large to inclose the younger ones and overtop the stem tip gives a simple measure for comparisons. Although this method has the obvious defect that a longer apex will need a rather more mature leaf to inclose it, this is not serious because the leaves are elongating rapidly by the time they are forming the "hood" and leaving the immediate region of the apex.

rapid elongation of the apex and inflorescence initiation, when buds develop precociously up on the apex itself and the early growth of the primordia becomes slower, so that the buds (in reality axillary buds) become the conspicuous feature and the subtending primordia in whose axils they arise no longer attract attention (fig. 1*E*, *F*, *F'*).

Underground buds grow out into new rhizomes. Under favorable conditions, the next one (or sometimes two) in the axil of the lowest aerial leaf produces a flowering shoot in the same way as the main axis. Of the remaining buds, only the lowest two or three grow out and then produce only "blind" shoots. Their axes elongate and the shoots are elevated

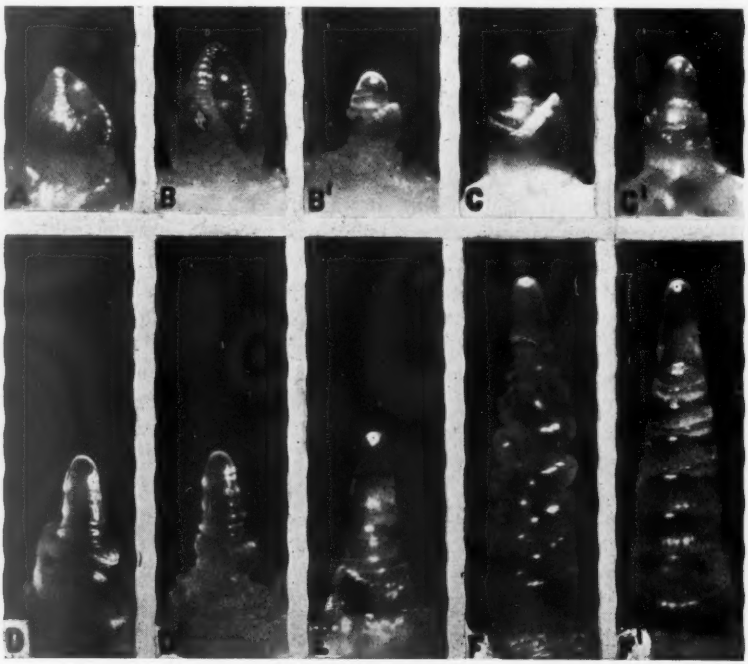


FIG. 1.—Dissected apices, mid-April to end of May (*A-C'*, vegetative; *D-F'*, just prior to and after inflorescence initiation). *A*, apex from rhizome with outer scale leaf and next four in the bud removed. *B*, from shoot with fourth leaf lamina fully exposed. *B'*, same with next outer cowl-like leaf (eighth on shoot) removed. *C*, similar apex from slightly more mature shoot. *C'*, same apex with next leaf (ninth on shoot) removed. *D*, apex just prior to inflorescence production from shoot with seventh leaf lamina fully exposed. *D'*, same with next leaf (twelfth) removed. *E*, apex at inflorescence initiation from shoot with seventh (? eighth) fully exposed leaf and four others in bud removed. *F*, *F'*, two views of young inflorescence with seventh (? eighth) fully exposed leaves and four others removed. See text for method of numbering.

Buds normally occur in the axils of the scale leaves and the first five green leaves. In dissected material they are not seen externally until the primordium has grown well over the top of the apex. rather like the flowering shoots, but only a succession of leaves is developed. The apex of such shoots is small, possibly smaller than that of a rhizome, and the rate of leaf initiation seems slow. In dis-

sected material the young primordia always seem pliant and soft, as though suffering from lack of water.

Developmental anatomy

Apex.—Clothing the tip of the apex is a single layer of anticlinally dividing cells—the dermatogen (figs. 2; 3; 5; 7*A*). Although—in view of what will be shown to be its role in leaf initiation—this layer cannot of course be regarded as a "dermatogen" in Hanstein's original sense, it seems desirable to keep his term, without, however, letting it carry his implications.

Disregarding for the moment the ultimate origin of the tissues, a little way back from the apex they are found to be arranged in a definite and characteristic pattern. The outer cells are dividing mainly anticlinally and so giving rise to a number of strata. The layer immediately inside the dermatogen will be referred to as the hypodermis (*h*, figs. 2–6*A*; 7*A*; 9).

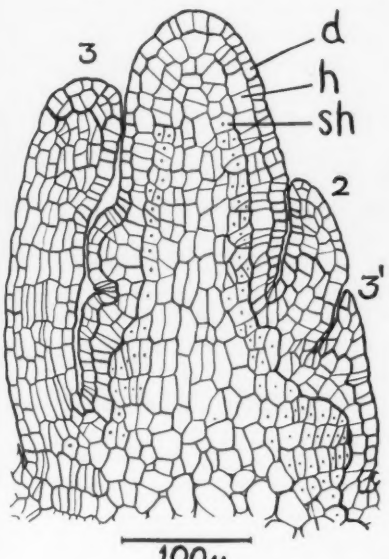


Fig. 2.—Radial longisection of apex of rhizome in plane of leaves: 2 and 3, tips of second and third primordia; 3′, encircling base of third primordium, counting from tip. *d*, *h*, *sh*, dermatogen, hypodermis, and subhypodermis.

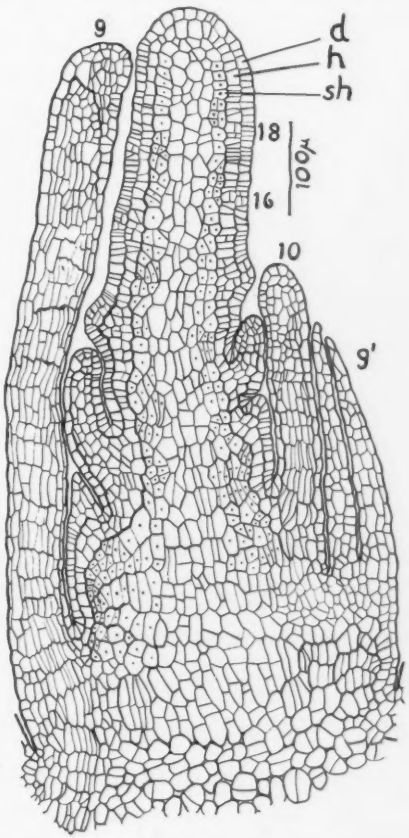


Fig. 3.—Radial longisection (in plane of leaves) of apex of shoot with laminae of about five leaves exposed. As mature shoots normally bear only twelve green leaves in all, only the basal four primordia would develop to completion and the higher ones would cease growth early. 9′, two encircling overlapping wings of base of ninth leaf. *d*, *h*, *sh*, dermatogen, hypodermis, and subhypodermis.

Beneath this is a layer designated the subhypodermal layer (*sh*, figs. 2–6*A*; 7*A*; 9). In all the figures most of the cells of this layer are indicated by dots, intended to show the outer limits of this layer and

distinguish between it and the hypodermis. These dots are not intended to define the inner limits of the subhypodermis: where the distinction is clear, as in most cases, all the layer is dotted, but where the exact inner boundary is indefinite (as, for instance, toward the base of figures 2 and 3 or in figure 4*K*) only the outer cells are marked. In some figures it will be seen that quite a few more cells can be assigned to this layer, but it was thought better to limit the dots to the very obvious.

Inside the subhypodermal layer is a central core of rather larger, less rapidly dividing cells with less densely staining contents, forming what might be described as an "apical pith." When much starch is present, as in the rhizome, these central cells have an abundance, while only a few grains appear in occasional cells of the outer three layers. In the young and the old aerial shoots—and the rhizome as well—the part of the apex inside the dermatogen "shell" seems to originate from two initials (or groups of initials), one lying vertically above the other. This situation represents that described by RÖSLER (18) and KLIEM (15) for the older apices of *Triticum* and *Avena*, respectively. In these two species, at inflorescence production the regions called in the present study subhypodermis and core are derived from an initial or group of initials, which for long periods is distinct from that above, which is solely giving rise to the hypodermis. Both RÖSLER and KLIEM make a great point of showing that this condition in *Triticum* and *Avena* is only temporary because every now and again the hypodermal initial divides periclinally, resulting in the displacement backwards of the old initial of the "subhypodermis-plus-core" and its subsequent loss into the differentiating tissues leaving the apical region. In *Agropyron*, however, in both the rhizome and the aerial shoot, careful examination of the median sections of series of longitudinal radial sections cut in the plane of the leaves failed to reveal periclinal divisions in the initial cell or cells of the hypodermis at the apex. The hypodermis appeared as a single unbroken layer over the apex, unless the section was off the median—when it was possible to obtain a spurious effect of periclinally divided hypodermal cells where the files running back from the apex were cut at an angle. Thus it would seem that in *Agropyron* periclinal divisions of the cells at the tip are extremely rare, so that the hypodermis approaches a morphological layer.

This difference between *Agropyron*, and *Triticum* and *Avena* may be related to the dimensions of the apex: the vegetative apex of *Agropyron* is a more massive structure and therefore may have a type of growth more related to that seen only in later stages in *Triticum* and *Avena*, when their apices have increased in bulk. It is conceivable that the interplay of factors like cell size and apical dimensions may have considerable bearing on the particular scheme followed by a given species at a particular period in its ontogeny. For this reason the question of the origin of the particular layers is not pursued further.

To summarize: the apex consists of an inner core of rather slowly dividing cells inclosed in three thimble-shaped "shells"—the subhypodermis, the hypodermis, and the dermatogen, the cells of each shell (especially those of the dermatogen) dividing mainly anticlinally. The two outer shells, the dermatogen and the hypodermis, seem to originate from separate initials or initial groups, while the subhypodermis and core are derived from a common initial or initial group.

ORIGIN OF PRIMORDIA.—In longitudinal radial sections taken in the plane of the leaves, the initiation of a new primordium is usually indicated by the appearance of periclinal divisions in one or two cells of the hypodermal layer (figs. 3; 4*A*; 6*A*) which are immediately followed by periclinal divisions in the dermatogen (figs. 2; 3; 4*B*; 6*A*, *B*). This stage is seen in transverse section in figures 5*A*, *B*; and 7*A*. It is difficult to be absolutely certain whether the cells of the hypodermis divide periclinally before those of the dermatogen, or whether the two are simultaneous. In longitudinal sections it is often possible to find divisions in the hypodermis foreshadowing the future primordium without being able to find any in the dermatogen, either in the median sections or in those on each side of them. In transverse sections, examination of a number of series did not reveal a single case where the hypodermal cells had divided without a single dermatogen cell doing likewise. Figures 5*A* and 7*A*, with a divided dermatogen cell on the flank of the future primordium, are typical of what was observed. Sections each side of that illustrated showed that this was the only dermatogen cell which had divided periclinally at this level. The fact that so many of the hypodermal cells have divided periclinally suggests that at some time earlier some of them would have divided but all the dermatogen cells would still be dividing only anticlinally. In cases like that illustrated, the sections higher up the apex (where the next higher primordium is to appear) do not throw any light on the matter, because so near the tip as this the core, subhypodermis, and hypodermis are not easily distinguished.

In *Agropyron* the primordium is usually initiated by periclinal divisions in two dermatogen cells only, when seen in longitudinal section (from about two rows of dermatogen cells placed horizontally over each other). RÖSLER (18) suggested a depth of three cells for *Triticum*, but KLIEM (15) stated that in *Avena*, although the primordium may arise occasionally from a depth of three dermatogen cells, it usually comes from only two, and may even be derived from a single row. He elaborates the point by illustrating the slight differences in cell patterns which follow the three alternatives, and designates the origin from a depth of one, two, or three dermatogen cells as types A, B, and C, respectively. This, however, seems to be laboring a rather simple matter, since the number of cells involved will probably largely depend on their actual dimensions at the point where the future primordium is to arise. For example, two daughter cells resulting from a recent anticlinal division would probably both divide periclinally, whereas a large dermatogen cell might tend to divide periclinally first and then the two daughter cells divide anticlinally.

Although the first dermatogen cell to divide periclinally may not be situated at the center of insertion of the future primordium, and even the first two cells to divide may be far apart on each side of the future middle line, later activity soon produces a sector of periclinally dividing cells, with divisions most rapid in the middle of the primordium (figs. 5*B*, *C*, *D*; 7*B*, *C*). This would suggest that some internal stabilizing influence is at work insuring that the new primordium continues the strictly distichous arrangement of the leaves.

ENCIRCLING GROWTH OF PRIMORDIUM.—The rapid lateral spread of the periclinal type of division in the dermatogen and hypodermis is responsible for the transformation of the original rather

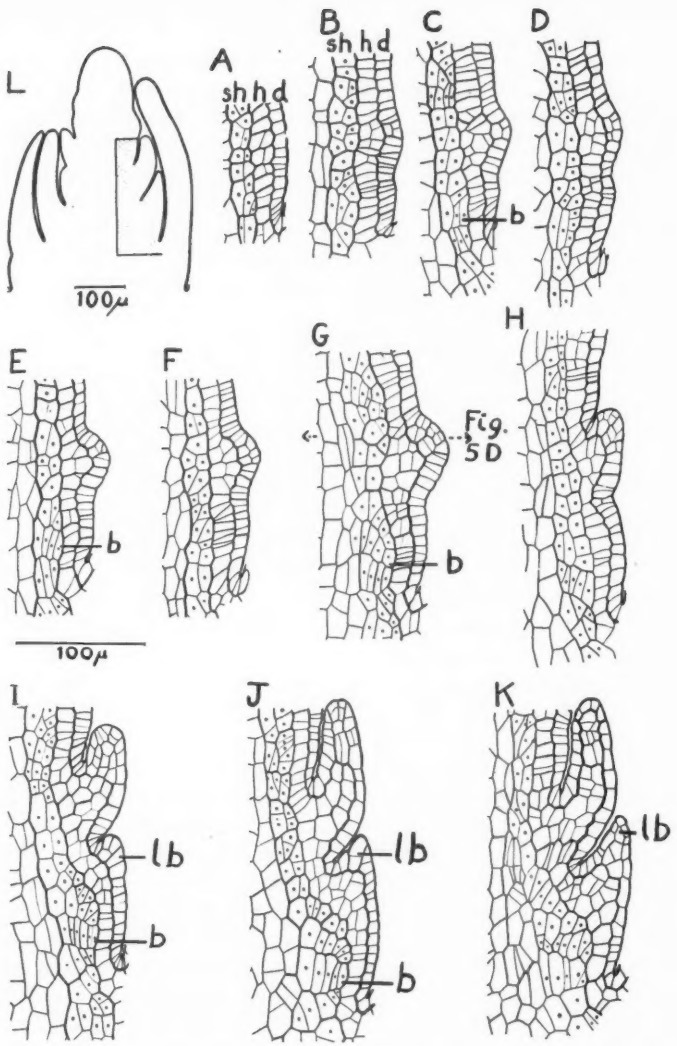


FIG. 4.—Radial longisections in plane of leaves showing stages in initiation of primordium on side of apex. *b*, cells which foreshadow bud in axil of leaf lower down; *lb*, base of primordium growing around from other side of axis. *d*, *h*, *sh*, dermatogen, hypodermal and subhypodermal layers. (Dots employed to indicate subhypodermal layer are purely conventional and in later stages are not intended to define absolutely the inner limits of this layer.) *L*, longisection of whole apex showing position and extent of tissue included in *K*.

local protuberance into a crescent and then into a collar. Because the leaves are in two ranks, in radial longitudinal sections the encircling growth of those on one side of the apex begins to show in the positions alternating between the insertions of the primordia on the other side (figs. 2, 3). In figures 4*I* and *J*, for example, the conditions of the cells immediately above and below the insertion of the primordium represent the stages reached by the encircling growth of the leaves inserted on the other side of the axis above and below the primordium actually figured. Thus, for convenience of illustration, for each primordium only it itself plus a small portion of the axis above and below are shown, rather than the whole section actually concerned—or the "disc of insertion" as BUGNON would call it. The portion illustrated is indicated in figure 4*L*, and reference to the longitudinal sections of complete apices in figures 2 and 3 may help in this matter.

The periclinal divisions in the cells of the hypodermal layer in a position on the axis below the primordium in figure 4*D* and the divisions above and below in figure 4*E* indicate the spreading-around of the insertion of the leaves on the other side of the axis. Whether the first indication of this spreading comes early (as in fig. 4*E*) or late (as in fig. 4*H*) depends on the actual apex concerned. Usually, some time after the hypodermal cells have divided periclinally the dermatogen cells do the same (figs. 4*H*, *I*, *J*; 6*E*, *F*, *G*), so that the two layers become responsible for the tissues of the up-pushing which marks the encircling base of the leaf insertion on the other side of the axis. This is clearly seen in the case of primordium 11 in figure 3.

In the apices of rhizomes and young aerial shoots the two encircling edges of the primordium meet at the same level; but in older shoots apparently the two sides overlap, giving the appearance shown for primordium 9′ in figure 3. This seems to be accomplished on one side by the outer wing continuing its encircling growth by dermatogen (and hypodermal?) divisions on the outer face of the future inner wing, and presumably the inner wing doing the same by divisions on the inner face of the outer wing on the other side of the mid-line. Thus the future sheath is tubular at the extreme base but has two overlapping edges higher up. No doubt, differences in the rate of encircling growth cause differences in the degree to which the sheaths appear "split" or "fused" in various grass species (see ARBER [2], quoting for *Festuca* from HACKEL). It is often difficult to picture exactly what happens during the development of the overlapping portions, but some similar course as this probably explains the condition in *Zea*, where the overlap at the insertion may be as much as half the circumference of the stem.

There seems little doubt that the hypodermal cells contribute to the tissues of the lower part of the primordium, as suggested by BUGNON and KLIEM. The primordia shown in figures 4*K* and 6*E*, *F* seem to permit no other explanation. Cleared apices also suggest that the primordia are not derived solely f om the dermatogen, as reported by DOULIOT (12), whose work was confined to hand sections, and by RÖSLER (18), who conceived that the very young primordium (*Blatthöcker*) arose from the dermatogen alone but seems to have left open the question of its later development.

During the early growth of the primordium the cells throughout divide actively, and rapid increase in its length is due to the fact that most of the divisions are at right angles to the long axis,

giving files of cells in the interior and at the surface of the leaf. This is a striking feature when grass shoots are being dissected under a binocular microscope, the shining facets formed by the convex outer walls of the epidermal cells forming lines of bright dots. Occasional longitudinal divisions contribute to the width of the leaf, causing the appearance of two files of cells in continuity with only one at the top and bottom. Occasional longitudinal divisions parallel to the future upper and lower surfaces lead to the increasing thickness of the young leaf.

The cells of the lower (abaxial) surface divide more rapidly and at the same time become more elongated than those on the inner surface, with the result that the primordium assumes a vertical position (fig. 4*G–J*) and so becomes converted from a ring into a small collar (fig. 1).

For a considerable time, usually until the primordium is about to overtop the apex, new tissue is being added to the interior by the cells at the free edge and tip. This is indicated by the periclinal divisions in the cells shown at the tip of the primordia in figure 4*H*, *I*, *J*, and in the ninth and eleventh primordia of figure 3. Thus the whole of the small mass of tissue illustrated in these figures has been derived lately by apical activity, and the original limits between the cells derived from the dermatogen and hypodermis are much lower, probably about half-way down the primordium.

At the same time, the cells at the margins of the primordium are also adding to the internal tissues at the wings and appear as two continuous bands running from the tip to the insertion. They are behaving in the same way as the initials at the tip and are not really separable from them, as the whole forms what is originally the free edge of the ring or collar and later the tip and sides of the cowl as the primordium changes its shape. This is very striking in cleared material.

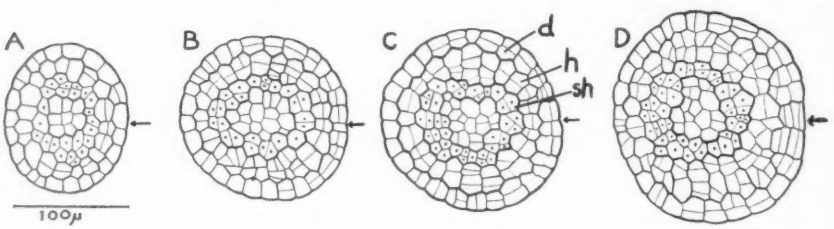


FIG. 5.—Transections showing stages in initiation of primordium (arrows indicate mid-line of future leaf). *D* is taken at about level indicated in fig. 4*C*. *d*, *h*, and *sh*, dermatogen, hypodermis, and subhypodermis. Dots used to indicate subhypodermal layer are conventional and not intended to imply any noticeable difference in contents.

When the young leaf is beginning to inclose the younger primordia and the apex, the cells in the position of the future midvein start to divide longitudinally, the median provascular strand is formed in the leaf and axis, and then the primordium rapidly overtops the apex and begins to leave the apical region. At about this time the ligule arises by periclinal divisions in two or three horizontally running rows of cells of the epidermis of the adaxial surface, exactly as described for *Zea* (**21, 22**).

ORIGIN OF AXILLARY BUDS.—So far little mention has been made of the axillary buds, although their first stages are evident very early. When periclinal di-

visions in the dermatogen and hypodermal layers mark the initiation of a new primordium, the cells of the underlying subhypodermal layer themselves divide once periclinally and usually only rarely divide in that plane again until the primordium has become a distinct collar (figs. 3; 4*A–I*). However, they do divide actively just above the primordium, that is, in a position just below the encircling insertion spreading around from the leaf situated above on the opposite side of the axis. They are marked *b* in the figures.

The more central cells of this new activity in the subhypodermis divide periclinally, but those immediately above and below divide by walls which are curiously half periclinal and half anticlinal (fig. 4*G*). This results in a tissue composed of cells with walls which all appear to be on the circumferences of circles whose center is the position of the future bud (figs. 4*C, E, I, J;* 6*D, F, G* at *b*). It seems that the tissue is derived from a vertical depth of four or five cells, although the more central two or three are the most active.

In transverse section this tissue appears as a few radially running files of cells (fig. 7*D–F*). Figure 8*A* represents a transverse section of about the stage seen in the lower part, at the level *b* in figure 4*A*. Figure 8*B*, *C*, and *D* show stages comparable with those seen in the lower part of figure 4*C*, *D*, and *E*, respectively, while figure 8 *E–G* represent a close series of stages at about the maturity shown in figure 4*G–J*. Figure 6*H* is probably comparable with figure 4*K* or figure 9*A* or *B*.

To link up these sections of bud stages with those illustrating the initiation of the leaf primordium, figure 8*A* can be regarded as about the condition to be found in sections immediately below the one illustrated in figure 5*A*. Figure 8*B* or *C* would come under that shown in figure 5*B;* that of figure 8*D* would come under that of figure 5*C;* and the stages in figure 8*E* or *F* would probably be found under such a stage as that shown in figure 5*D*.

In transverse sections in the region of the future bud, the cells of the hypodermis are often seen to be divided half anticlinally and half periclinally, so that the planes of their new walls (in cells *x* in figure 8*C* and *D*) are more or less parallel to those of the files of cells in the subhypodermal layer. It is rather as though the divisions were spreading across from the subhypodermal layer into the hypodermis at the sides. The cells of the hypodermis a little nearer the center of the future bud tend to divide (fig. 8*D*, *y*), and emphasize the rather bowl-shaped appearance of the tissue in this region. At the center there seems to be one or more hypodermal cells which divide only periclinally (figs. 4*I, J;* 7*G–I;* 8*E–H;* 9*A*). Presumably these are destined to be the initials of the hypodermis of the future bud apex.

In figure 8*G* and *H* only the more central cells intimately connected with the bud are dotted; their inner limits are not suggested as they are difficult to determine. Although in the region of the future bud it is the subhypodermal cells which divide most actively, at the sides of the axis the hypodermal cells are the most active (fig. 8*D–F*). Thus although the inside of the subhypodermis is more or less circular in cross-section, the outer limits are oval. In figure 8*G* and *H* the tissue on each flank is undoubtedly derived from the hypodermis, although it is not certain to what depth it extends in these stages. This lateral activity of the hypodermis is also visible at the level of the middle of the primordium (fig. 5*B*, *C*).

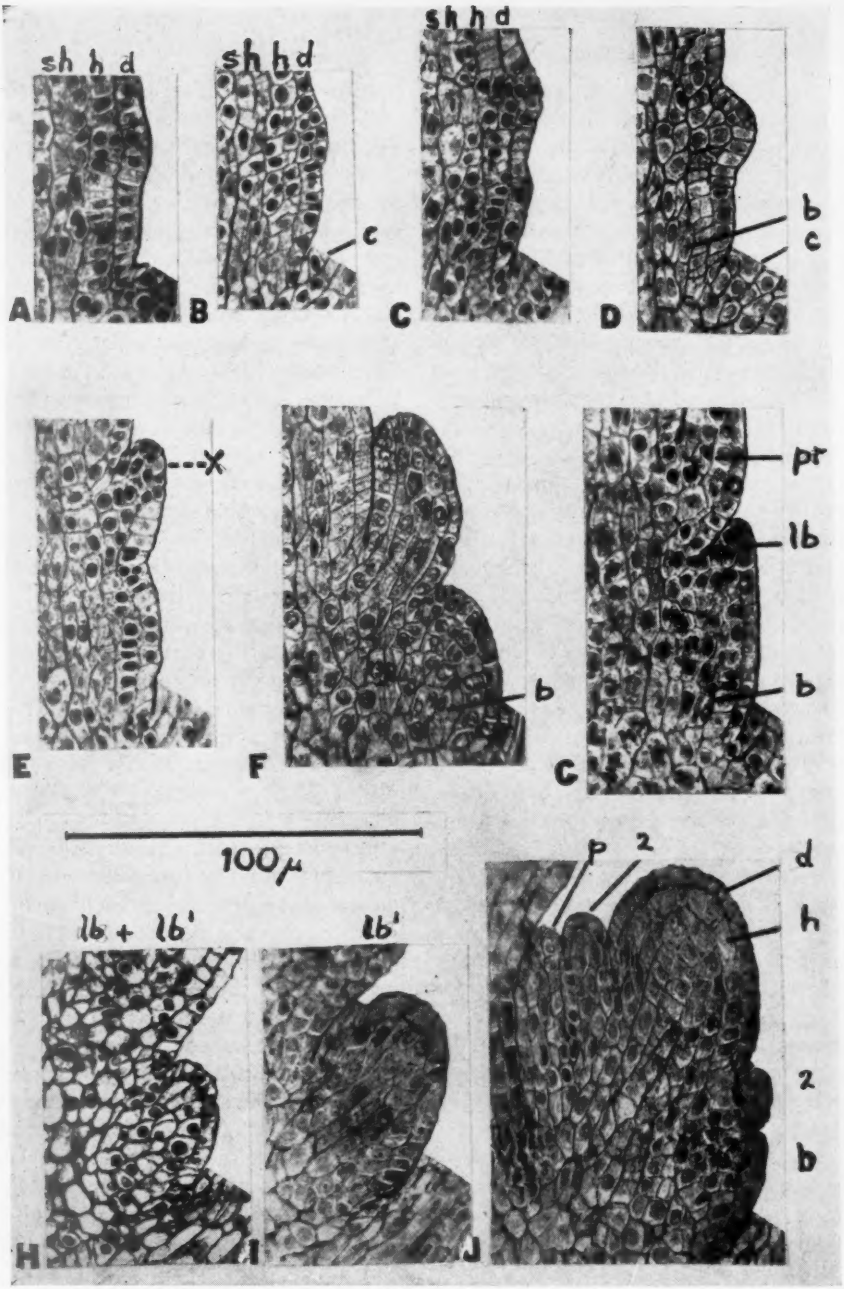


FIG. 6.—Radial longisections. *A–F*, in plane of leaves, showing initiation of primordium on side of apex. *d*, *h*, *sh*, dermatogen, hypodermis, and subhypodermis. *c*, base of leaf (removed) inserted directly below; *b*, cells which foreshadow future bud. *G*, section through base of primordium (*pr*) and encircling insertion (*lb*) of next oldest leaf growing around from other side of axis. *H–J*, sections in plane of leaves of main axis showing development of axillary bud. *d*, *h*, dermatogen and hypodermal layers; *p*, prophyll; *2*, second leaf of bud; *lb*, *lb'*, two overlapping wings growing around from primordium above on other side of apex.

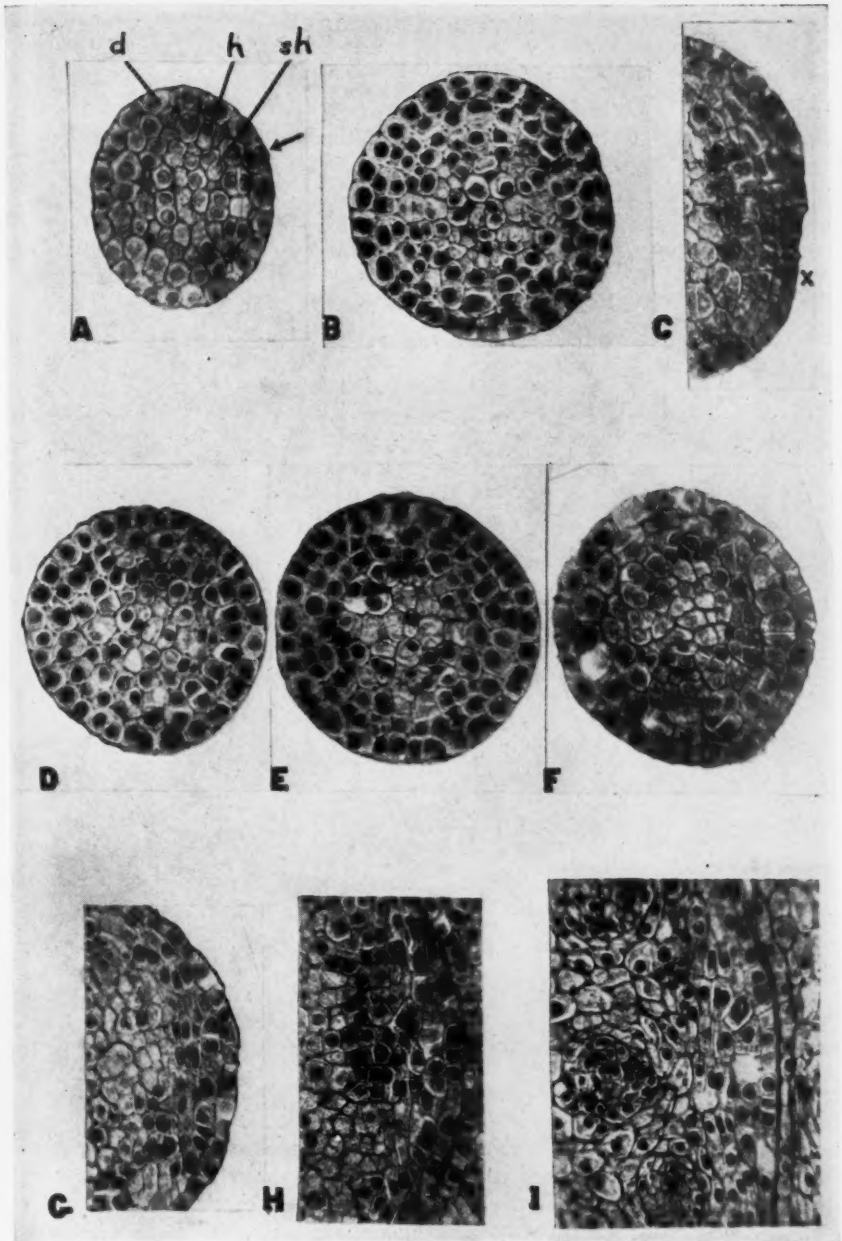


FIG. 7.—Transections of axis. *A–C*, at point of initiation of leaf primordium. (Arrow in *A* indicates first periclinal division which could be found connected with future primordium, although in this and other sections a number of hypodermal cells have already divided periclinally.) *C*, taken at level (*x*) shown in fig. 6*E*, showing further divisions of dermatogen cells (at *x* it would seem that one of the inner daughter cells divided anticlinally before dividing again periclinally). *d*, *h*, *sh*, dermatogen, hypodermis, and subhypodermis. *D–I*, stages preceding development of axillary bud: *D* at about same stage as that in fig. 6*C*; *E* and *F* at about that in fig. 6*D*; and *G–I* between that of fig. 6*G* and 6*H*.

At about the stages represented in figures 4*J* and *K*; 8*F* and *G*; and 9*A*, the cells at the outside of the central files seem to have divided more actively and are often smaller, with rather more densely staining contents. They are presumably the future initials of the subhypodermis and core of the bud axis. to be situated. This view is also supplemented by the way in which its subsequent behavior is correlated with events in the leaf above rather than in the one below (22).

EMERGENCE OF AXILLARY BUD.—Figures 6*G–J* and 9*A–I* are of radial longitudinal sections in the plane of the

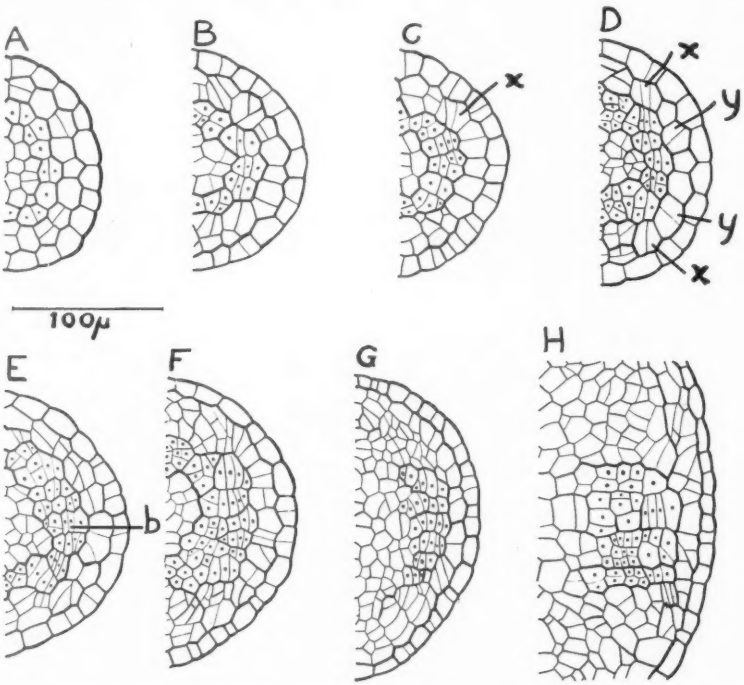


FIG. 8.—Transections showing preceding stages in development of axillary bud. *b*, files of cells shown in longisection in fig. 4. Dots used to indicate subhypodermal layer are conventional and the limits of only part of this layer are suggested in *G* and *H*. *x*, *y*, divisions in hypodermal cells referred to in text.

The position of the files of cells foreshadowing the axillary bud is interesting because of the manner in which it is so intimately bound up with the development of the encircling growth of the leaf above and on the other side of the axis. This suggests that the bud should be associated with that leaf rather than with the one in whose axil it will later appear leaves of the main axis and show stages in the emergence of the axillary bud. It will be seen that at the beginning a vertical depth of four or five files of subhypodermal cells (dotted) is involved, but as development proceeds the tissue of the inner part tends to be confined to about the middle three files—about nine to twelve cells in cross-section of the new

bud axis. The cells derived from these files undergo further divisions, so that there appear to be up to about six files at the base of the bud in longitudinal section (fig. 9*F–H*). In figure 9*A–C* the hypodermis above the bud has increased in width where the insertion is coming around from the leaf above; figure 9*J*, which shows the position of the bud in figure 9*E*, helps to interpret the relationships of the structures at this stage.

The tissues just behind the bud are penetrated by the median provascular strand to the leaf but one above (to the next leaf inserted on this side); such a stage is shown in figures 7*I* and 9*C*. Lateral provascular strands to the insertion of the primordium of the leaf above (inserted on the other side of the axis) are differentiating out of the tissue at this stage. Possibly this differentiation of provascular strands in the "parent"

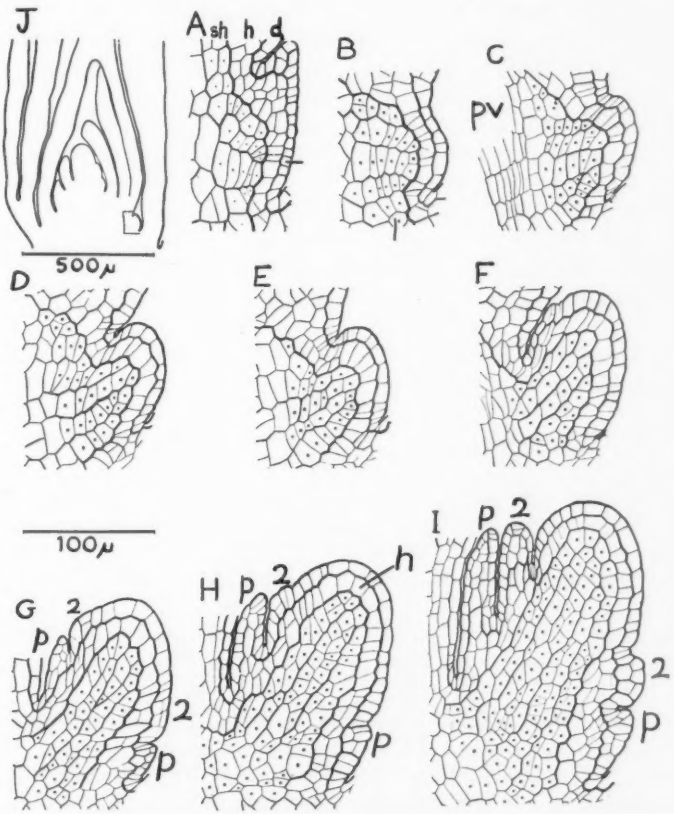


FIG. 9.—Radial longisections in plane of leaves on main axis showing development of axillary bud. *d*, *h*, *sh*, dermatogen, hypodermis, and subhypodermis. *lb*, encircling base of leaf above and on other side of axis. *p*, prophyll of bud; 2, second leaf of bud; *pv*, provascular strand in main axis. Dots in subhypodermal layer are not intended to define inner limits of this layer. *J*, longisection of whole apex showing orientation of tissue drawn in *E*.

axis is correlated with emergence of the bud.

The bud begins to grow outward mainly by the activity of the subhypodermal cells. At the same time it takes a course upward, so that its own axis is at an angle of about 45° with that of the "parent." This is brought about by the more rapid divisions in the files of subhypodermal cells on the lower side. About the time the bud begins to grow out, periclinal divisions take place in the hypodermis on the upper side of the bud (fig. 9*E*), followed by divisions in the dermatogen (fig. 9*F*), indicating the initiation of the first leaf of the bud—the prophyll. Sooner or later other traces of it may be seen below the bud, as in the divisions of the hypodermal cells in figure 9*F* and those in the hypodermal and dermatogen cells in figure 9*G*, *H*. Later, as at "*2*" in figure 9*G*, *H*, and *I*, parts of the second bud leaf appear. In the case of the leaves on the bud, the parts appearing on each side of the axis seen in these sections are the insertions spreading around the bud axis. A longitudinal radial section of the main axis in the plane of the leaves cuts the bud axis at right angles to the plane of its leaves. Thus a section of a bud which is fairly well grown out, as in figure 9*I*, shows the conditions obtaining at right angles to that illustrated in figures 2 and 3. Of course, it is rarely possible to go exactly vertically down the whole bud axis, hence some of the files seen in figure 9*I* are not complete from the base to the top.

No periclinal divisions are found in the dermatogen at the position of the tip of the future bud, neither during the early stages of the initiation nor at any subsequent time during its emergence (figs. 6*G–J*; 9*A–C*). Although periclinal divisions play their part in the production of the prophyll (fig. 9*F*), the dermatogen contributes nothing to the internal tissues of the axillary bud apex.

As is evident from the figures, the divisions in the hypodermal layer at the tip of the bud have all been anticlinal. Careful examination of numerous series showed—provided one was dealing with the center of the bud—no periclinal divisions. Of course, sections off the median showed such divisions in the hypodermis *apparently* at the tip, but in such cases one is looking at the divisions in the position of the next primordium.

RÖSLER (**18**) and KLIEM (**15**) stated that, in *Triticum* and *Avena*, respectively, the new bud apex grows by only two sets of initials, one for the dermatogen (tunica) and the other for the internal tissues (corpus), thus following the mode of growth of the parental apex. RÖSLER figures periclinal divisions in the cells of the hypodermis at the tip of the emerging bud apex about the time the prophyll is being initiated a little farther back (**18**, fig. 15*g* and *h* and plate IV, fig. 39). This would mean that although the "core-plus-subhypodermis" of the new bud starts by being derived from the subhypodermis of the parent, it is later extended by tissue being cut off from the hypodermal initial or initial group. Assuming that this appearance is real and not caused by the examination of sections slightly off the median, it may well be that in both *Triticum* and *Avena* the bud apices resemble the main shoot in having less massive apices than *Agropyron* and consequently a more "juvenile" type of growth. In RÖSLER's figure 39 the bud apex has a width of six cells, which would mean about twenty-seven cells in a transverse section of its axis. In the comparable apex of *Agropyron* (fig. 9*H* or *I*) there are eight or nine cells in

its width, making about fifty to sixty cells in cross-section.

This difference in size of the emerging apex seems to be supported by differences in the early stages in the subhypodermis. In *Triticum* and *Avena* a vertical depth of only about three subhypodermal cells (RÖSLER fig. 15*a–d;* KLIEM fig. 30) and in *Triticum* a tangential width of about two to three cells foreshadow the future bud. When the bud actually begins to emerge, in *Triticum* (and possibly in *Avena*) the derivatives of a vertical depth of only two cells often play a part in the formation of the early core. In *Agropyron*, on the other hand, a vertical depth of four to five cells (figs. 4*I–K;* 9*A, B*) and a tangential width of three to four cells (fig. 8*H*) foreshadow the future bud. In the core of the emerging bud the derivatives of a vertical depth of three to four cells are actually represented (fig. 9*D–G*). Thus in the solid, in *Triticum* (and presumably in *Avena*) the early core-plus-subhypodermis of the bud is composed of files of cells derived from about four to six of the original subhypodermal cells, and in *Agropyron* it is made up of nine to twelve similar files.

From about the stage shown in figure 9*H*, the center of the bud axis begins to develop as a distinct core (fig. 9*I*). The central tissue originally derived from the subhypodermal layer of the parent axis is being delimited into an outer shell (the new subhypodermis of the bud) and an inner core two or three cells in diameter at the top. Thus the new core of the bud is not derived from the core of the main axis but from the "parental" subhypodermal layer. At the stage represented in figure 9*I* the apex appears to be growing in the same way as the parent: separate initials or initial groups are probably present for (*a*) the dermatogen, (*b*) the hypodermal layer, and (*c*) the new subhypodermal layer and the core within.

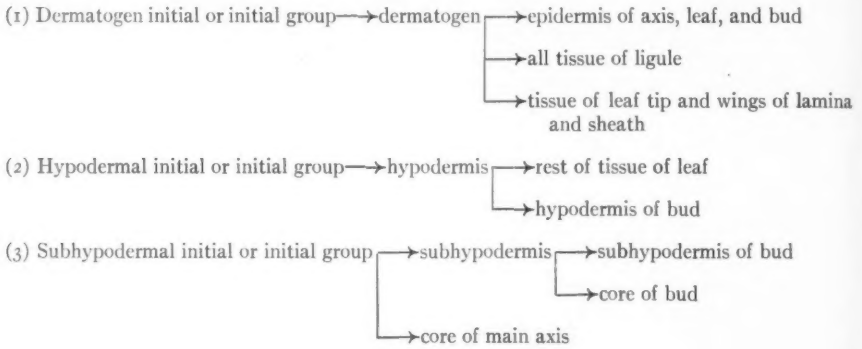
Deductions and inferences

APEX, LEAF, AND BUD PRIMORDIA.—In all of more than twenty species of grasses which the writer has examined, the conditions are substantially as those described for *Agropyron*. Always the dermatogen and the hypodermis have been concerned in leaf production and the subhypodermal layer called into play at bud formation. This is not taken to mean that these three layers have any phylogenetic importance, or that they are to be considered too rigidly significant. It is conceivable, for instance, that in some particular species the leaves may be derived solely from the dermatogen, or in some other "atypical" way. The real significance would appear to be that the normal leaf is of relatively superficial origin, while the axillary bud incorporates deeper tissue. The summary on page 286 of the mode of leaf and bud initiation in *Agropyron* probably holds good for the majority of the Gramineae, and, moreover, is probably correct for the modified leaves like glumes, etc., as well.

In a young grass shoot, or where the apex is less massive, there may be only one common initial or initial group for both (2) and (3), but periclinal divisions in the cells cut off result in the production of a hypodermis and a subhypodermis which behave in the same way as above.

APPLICATION TO GRASS AND CEREAL BREEDING.—The mode of origin of the buds has a special significance in cereal and grass breeding techniques involving the use of colchicine to obtain amphidiploids. In the Gramineae the ovule is not derived from the carpel wall but from the residue of the axis on which the palea,

stamens, carpel, etc., are borne. The archesporial cell appears in the hypodermis and is apparently usually the initial cell of this layer; it gives the megaspore mother cell without further division. In the case of the stamen the situation is not so clear, for although the archesporial tissue is derived from the hypodermis of the young anther, it is not at all certain whether the anther itself is initiated as a normal leaf involving periclinal divisions in the dermatogen cells, or whether it is more in the nature of a bud


(1) Dermatogen initial or initial group
dermatogen
epidermis of axis, leaf, and bud
all tissue of ligule
tissue of leaf tip and wings of lamina and sheath
(2) Hypodermal initial or initial group
hypodermis
rest of tissue of leaf
hypodermis of bud
(3) Subhypodermal initial or initial group
subhypodermis
subhypodermis of bud
core of bud
core of main axis

—a situation which appears very likely, judging from its morphology in dissected material and the illustrations of similar material which have been published (**6–10, 16**). A preliminary study of *Anthoxanthum* seems to indicate that it is budlike and that periclinal divisions do not add to its inner tissues. A similar origin has been reported for the stamens of *Datura* (**19**).

Thus, if in a sterile hybrid viable pollen and ova are to be produced, the cells which must be made amphidiploid are those of the hypodermis. If at an early stage in the development of the seedling one of the hypodermal initials has its chromosome complement doubled, a sectorial chimaera will be produced, and then—supposing the doubled sector is in the plane of the leaves—all the buds on that side will have their hypodermis with the double number of chromosomes and will give viable gametes. The question of whether the flowers are borne on a tertiary or a quaternary branch or on one of any higher order has no significance. The younger the seedling when treated, the better, first because there are less initials to the hypodermis in young apices (RÖSLER and KLIEM), but mainly because young buds grow out into whole shoots and later inflorescences, while higher buds give a whorl of branches in the case of a panicle or a single spikelet in the case of a spike. Doubling the number of chromosomes in one or more hypodermal cells on the *side* of the apex will be effective in producing only one bud or a block of buds with the double complement.

As far as the breeder is concerned, it is of no consequence whether the hypodermis of the main shoot of his particular hybrid is produced by an initial or initial group of its own in the manner which seems to be followed in *Agropyron*, or whether it shares a common initial or initial group with the subhypodermis and core, which according to RÖSLER and KLIEM is effectively the case during the greater part of the life of *Triticum* and

Avena. Where the hypodermis does share the same initial or initial group with the subhypodermis and core, then doubling the number of chromosomes will cause all or a sector of the core cells to have the double number as well as the hypodermis. This may be an advantage for the future healthy development of the shoot, but it obviously does not affect the theoretical side of the question of the constitution of the axillary buds. Similarly, from the plant breeder's point of view, it does not matter whether the axillary bud grows with a common initial for the internal tissues in the manner suggested by RÖSLER and KLIEM for *Triticum* and *Avena*, or by separate initials for the hypodermis and subhypodermis-plus-core as described here for *Agropyron*, since in both cases the new hypodermis—the part which is important—has been derived directly from the hypodermis of the parent axis.

Doubling in the dermatogen will be useless as far as the axillary buds are concerned, but may perhaps be of some use if a considerable amount of the inner tissue of the stamen tips should be derived from it. Doubling of any initial or any of the cells in the subhypodermis or core will be useless unless some unusual development occurs later.

Discussion

In this study the description has been made purely objective, with the intention of avoiding all formal histogenic viewpoints and of avoiding also the introduction of theories about a determinate zonation of the apical tissues, either of the HANSTEIN or the BRESLAU types. Should it be argued that this is a sterilized outlook and that much is to be lost by the method, it may be defended that a formalized viewpoint is likely to obscure more facts that it reveals. To the writer the initiation of a primordium or a bud is not due to any inherent properties or "prospective values" of any layer, nor is it due to the cells concerned being derived from any special initial or initial group, but rather to a change in the type of metabolism of the particular cells involved; that is, an increase in their rate of protoplasm synthesis, rate and direction of division, etc. Since all the apical cells are fundamentally omnipotential, this change must be regarded as due to a change in their environment; that is, to a change in the direction, type, or speed of entry of "food," aeration, etc. The difference between the cells of the dermatogen and of the hypodermis, for example, is not inherent in the cells themselves, or due to their ultimate origin, but to what might be termed their position-metabolism. How the effects of differences in availability of "food" or water, changes in acidity or aeration, etc., are integrated is as yet totally unexplored; in fact, we do not even know whether a cell divides parallel to or at right angles to the resultant of all these factors. For instance, when the dermatogen cells begin to divide periclinally, has there been a swing around in the direction of the resultant of the influences at work on the particular cells?

The various layers of cells in the apex are to be likened to a gardener's topsoil and subsoil—differences in types of activity and not in original constituents. Rigid use of the HANSTEIN type of zonation, or even of the more elastic tunica and corpus scheme, will only help to cloud over the many difficulties underlying the true picture of the processes at work during the initiation of the organs. (It might be added that FOSTER [13, 14] has expressed similar views.) If the more fluid "metabolic" type of zonation is substituted, then—although the

depth of the various layers may be fairly constant for related plants—variation would be expected if a wide series of types were examined: hence the layers of two, three, four, etc., cells reported for the "periblem," or the layers of one, two, three, etc., cells called the tunica by others; hence also the difference in the number of these layers between the main and axillary shoots of the same species (25).

Again, this idea of a nonrigid "metabolic" differentiation of the apical cells makes intelligible the occasional occurrence of periclinal divisions in the dermatogen, independent of the production of any future primordium, as reported elsewhere for *Triticum* (18) and *Zea* and *Agropyron* (20, 24).[2]

Summary

1. The rhizome and early aerial shoot of *Agropyron repens* Beauv. each possesses a relatively short apex bearing only a few primordia. During the growing season the apex becomes more elongated and bears more primordia, which now show smaller steps in advancement. Later the apex elongates considerably and becomes transformed into the inflorescence. Of the axillary buds which grow out, the highest ones produce "blind" shoots, the lowest develop into shoots ending in inflorescences, and those underground give new rhizomes.

2. The shoot apex consists of three thimble-shaped layers—the dermatogen, the hypodermis, and the subhypodermis, which inclose a central core. There are probably three separate sets of initials or initial groups, one each for the dermatogen and the hypodermis and a common one for the subhypodermis and core.

3. Leaf primordia can first be detected by isolated periclinal divisions in the dermatogen cells on the side of the apex. These divisions are themselves probably preceded by similar ones in the hypodermis. Further divisions in the dermatogen and the hypodermal cells lead to the production of a crescentic protuberance. Increased activity in the region of the future mid-insertion insures the continued distichous phyllotaxis of the shoot. Lateral spreading of the divisions around the axis converts the crescent into a collar which then grows upward and produces a miniature cowl inclosing the apex and the younger primordia which have since arisen. Continued activity of the dermatogen at the free edge of the collar and then the cowl adds tissue to the tip and margins of the primordium. The internal tissue of the young leaf is derived from both the dermatogen and the hypodermis; the subhypodermis and core contribute nothing.

4. The axillary buds, which are foreshadowed by the production of radial files of cells in the subhypodermis, emerge soon after the insertion of the leaf above has spread around from the other side of the apex. No periclinal divisions occur in either the dermatogen or the hypodermis at the position of the future bud tip, so that these two layers in the bud are derived directly from the same layers in the main shoot. The subhypodermis and core of the bud are derived from the subhypodermis of the main axis.

5. The mode of origin and development of the axillary buds should be borne in mind when amphidiploids are to be produced by the action of colchicine on seedlings or young plants.

[2] Also, in a letter dated 10/3/41, Dr. A. S. FOSTER wrote: "One of my graduate students, working on *Triticum*, has found that periclinal divisions occur in the surface cells at the summit of the apices of seedling and *young* axillary buds."

6. It is suggested that the zonation observed in the apex is not due to any particular qualities inherent in the cells themselves but to the particular types of metabolism in these strata. This leads to a less rigid concept than that of either the HANSTEIN or the BRESLAU schools.

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DEVELOPMENT OF REPRODUCTIVE STRUCTURES IN THE BROWN ALGA TURBINARIA TURBINATA

H. L. BLOMQUIST

Introduction

Prior to the work of SIMONS (**23**) on *Sargassum filipendula* C. Ag., not much was known about the reproductive structures of the phaeophycean algae now included in the family Sargassaceae. SIMONS made two important contributions. First, she traced the origin of the cryptostomata and conceptacles to an epidermal initial cell, which in its first division by a downwardly curved, transverse wall gives rise to an outer, narrow cell, which she called the "tongue cell," and to a larger "inner cell." The latter she found to be the origin of the cells lining the conceptacle, which eventually bear the paraphyses, antheridia, and oogonia. Second, she observed a one-celled oogonium which was assumed to function directly as the egg without undergoing reduction division.

To check these observations, NIENBURG (**19**) investigated the conceptacle development of *Cystoseira barbata* (Godd. and Woodw.) C. Ag., and other genera of the order Fucales, and the oogonial development of *Sargassum linifolium* (Turn.) C. Ag. (**18**). NIENBURG agreed with SIMONS as to the origin and development of conceptacles in the representatives of the Sargassaceae which he studied, but he concluded that meiosis in *Sargassum* takes place in the oogonium and that this occurs after the oogonium is discharged and while it remains attached by a mucilaginous strand to the inside of the conceptacle. He also noted that meiosis is followed by another nuclear division resulting in eight nuclei, seven of which disintegrate within the cytoplasm of the egg. He found the development in *C. barbata* somewhat different, in that the oogonium remains *in situ* during meiosis and the seven supernumerary nuclei are extruded from the cytoplasm.

Since the completion of these investigations, considerable work has been done on other members of the Sargassaceae, especially by English, New Zealand, and Japanese investigators. Included in these studies are the genera *Bifurcaria*, *Carpophyllum*, *Coccophora*, *Cystophyllum*, *Cystoseira*, *Sargassum*, and *Turbinaria;* and the features which have received attention are: conceptacle development, oogonial maturation and discharge, cytology, and embryology.

While serving as an exchange professor at the University of Puerto Rico during the academic year of 1941–1942, an opportunity was afforded me for collecting material of the genus *Turbinaria*. This led to a study of the reproductive structures of this genus, which has received limited attention in previous studies.

In Puerto Rico, *Turbinaria* occurs most frequently on the northern or Atlantic side, where it grows on rocky substrata in two distinct locations. One is the eroded depressions in consolidated sandstone outcrops at or slightly above the mid-tide level, where the breakers strike the rocks with considerable force so that the spray or waves not only keep the depressions filled but by overflow maintain a constant supply of fresh water. Because of the low tides which prevail in the West Indies, these depressions are not often completely submerged, at least not to any considerable depth. In

such places the plants are rather small. Other algae associated with *Turbinaria* in these habitats are *Digenea simplex* (Wulfen) C. Ag., *Anadyomene stellata* (Wulfen) C. Ag., *Dictyosphaera favulosa* (C. Ag.) Decaisne, *Valonia ocillata* Howe, *Sargassum lendigerum* (L.) Kütz., etc. Other locations are in deeper water (3–5 feet) in more sheltered situations. Here the alga grows to larger sizes; one plant collected measured nearly 3 feet in length and weighed approximately 10 pounds. In these habitats it is usually associated with *Sargassum polyceratium* Mont., *S. platycarpum* Mont., *Bryothamnion triquetrum* (Gmel.) Howe, *Penicillus capitatus* Lam., species of *Galaxaura*, etc. To what extent it occurs at greater depths was not determined; but since it is not found attached in abundance in accessible places and yet whole plants and fragments are constantly being washed ashore, one may conclude that it occurs in considerable amount in deeper water.

The early taxonomic history of *Turbinaria* was reviewed by BARTON (2). According to her systematic treatment, eight species are recognized. DE TONI (8) listed nine species, while KJELLMAN (15) estimated three to five. On the basis of its general morphological features, it was early considered to be closely related to *Sargassum;* and in the establishment of the Sargassaceae, DE TONI placed it in this family.

According to BARTON, two species of *Turbinaria* occur in the West Indies. The most common of these is *T. turbinata* (L.) Kütz. The other, *T. tricostata* Barton, seems to be rare and may not differ sufficiently from the former to deserve specific rank (10). All the material used in this study has been referred to *T. turbinata*.

Material for imbedding in paraffin was fixed in chromic-acetic, formalin-acetic, or simply formalin. Plants were also pressed and dried for herbarium specimens.

The only morphological work dealing with *T. turbinata* was done by BARTON (2). Other species which have received attention by Japanese investigators are *T. fusiformis* Yendo (28), *T. ornata* J. Ag., and *T. filiformis* (12, 13, 14).

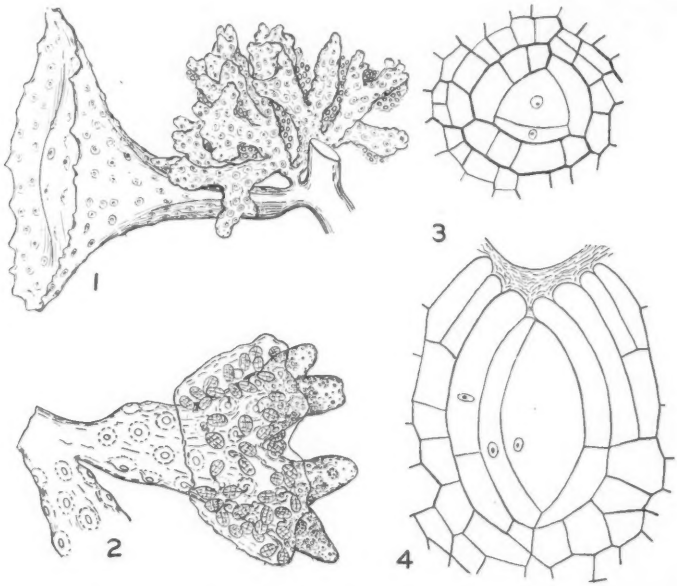
Observations

RECEPTACLES

The receptacles in *Turbinaria*, as in *Sargassum*, *Landsburgia*, and *Carpophyllum*, are axillary to the "leaves" or "phylloclads." They are richly branched, forming an irregular corymbose cluster (fig. 1). As was noted by BARTON, growth is initiated by a sunken apical cell which is triangular in cross-section (fig. 3). This type of initial cell is characteristic of the Sargassaceae. An exception seems to be *Carpophyllum flexuosum* (Esp.) Grev., in which DAWSON (3) found it to be truncated lenticular—as in *Fucus*. As to the shape of this apical cell longitudinally, there has been some uncertainty owing to the difficulty of obtaining strictly median sections or of knowing just when this was accomplished. After a careful study of transverse and longitudinal sections, it is concluded that in *T. turbinata* it is lenticular but may vary somewhat in symmetry (fig. 4).

In the branching of the receptacle, new apical cells originate from a preceding one, so that the tip of a receptacle shows a number of apical cells around a central one. As these are apparently formed in spiral succession, branching is fundamentally spiral, but this arrangement is more or less obliterated by irregularities in growth.

The genus *Turbinaria* has generally been considered strictly dioecious. While this may be true of *T. fusiformis* (**28**), *T. ornata*, and *T. filiformis* (**13**), a different situation is found in *T. turbinata*. Although plants are commonly of one sex, this species not infrequently shows hermaphroditic conceptacles. Also, occasionally both antheridial and oogonial conceptacles appear in one receptacle. BAKTON observed hermaphroditic conceptacles also in *T. conoidea* Kütz.



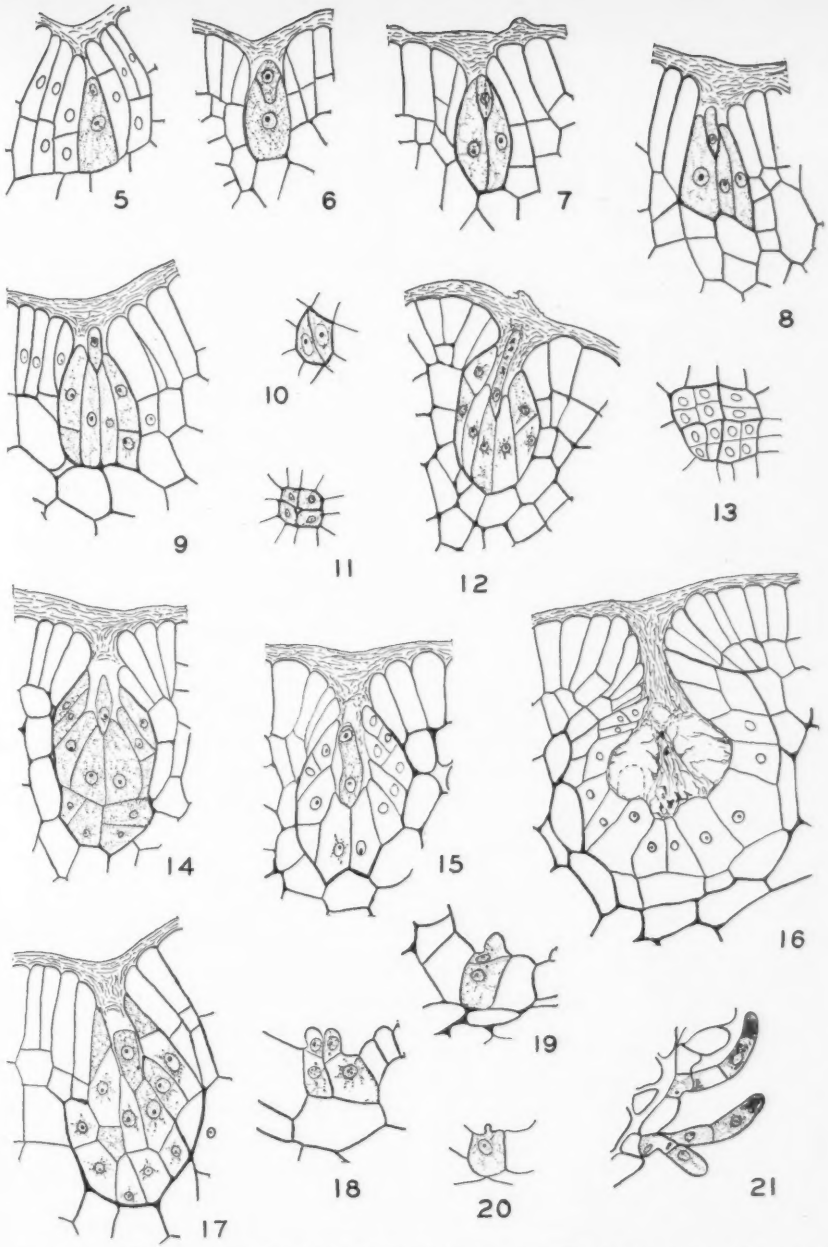
FIGS. 1–4.—Fig. 1, "leaf" with axillary receptacles. Fig. 2, receptacle with discharged oogonia in embryological development. Fig. 3, receptacle initial cell in transection. Fig. 4, receptacle initial in longisection.

CONCEPTACLES

Conceptacles originate from an epidermal cell which in longitudinal section is first distinguishable near a receptacle initial. It differs from the surrounding epidermal cells in its slightly depressed position, owing to the delayed first transverse division, and in its Erlenmeyer-flask shape (fig. 5). By a study of cross-sections of the tips of the receptacles, an attempt was made to determine how these conceptacle initials originate, but with inconclusive results. It must be assumed that they originate from successive segments of the receptacle initials, however, since the conceptacles appear to be spirally arranged—as was also observed by DAWSON (**3**) in *Carpophyllum flexuosum*.

The first division of the conceptacle initial is transverse by a downwardly curved wall (fig. 6), as in *Sargassum* and other genera of the Sargassaceae in which it has been observed.

The outer of the resulting two cells, the tongue cell, may develop into a paraphysis or may sooner or later disintegrate. In antheridial conceptacles the tongue cell usually develops into a nor-



FIGS. 5–21.—Fig. 5, conceptacle initial cell with divided nucleus. Fig. 6, first division of conceptacle initial forming tongue cell and inner or basal cell. Fig. 7, first division of basal cell. Figs. 8, 9, further divisions. Fig. 10, first division in transverse view. Fig. 11, second division in transverse view. Fig. 12, young oogonial conceptacle with degenerating tongue cell. Fig. 13, transverse view of stage of conceptacle shown in fig. 12. Fig. 14, young oogonial conceptacle with undivided tongue cell. Fig. 15, with two-nucleate tongue cell. Fig. 16, with disintegrating tongue cell forming mucilage. Fig. 17, young oogonial tongue cell undivided. Fig. 18, paraphysis initial cell with papilla. Fig. 19, papilla cut off from paraphysis initial. Fig. 20, two papilla developing adjacently. Fig. 21, "periphyses" below rim of ostiole.

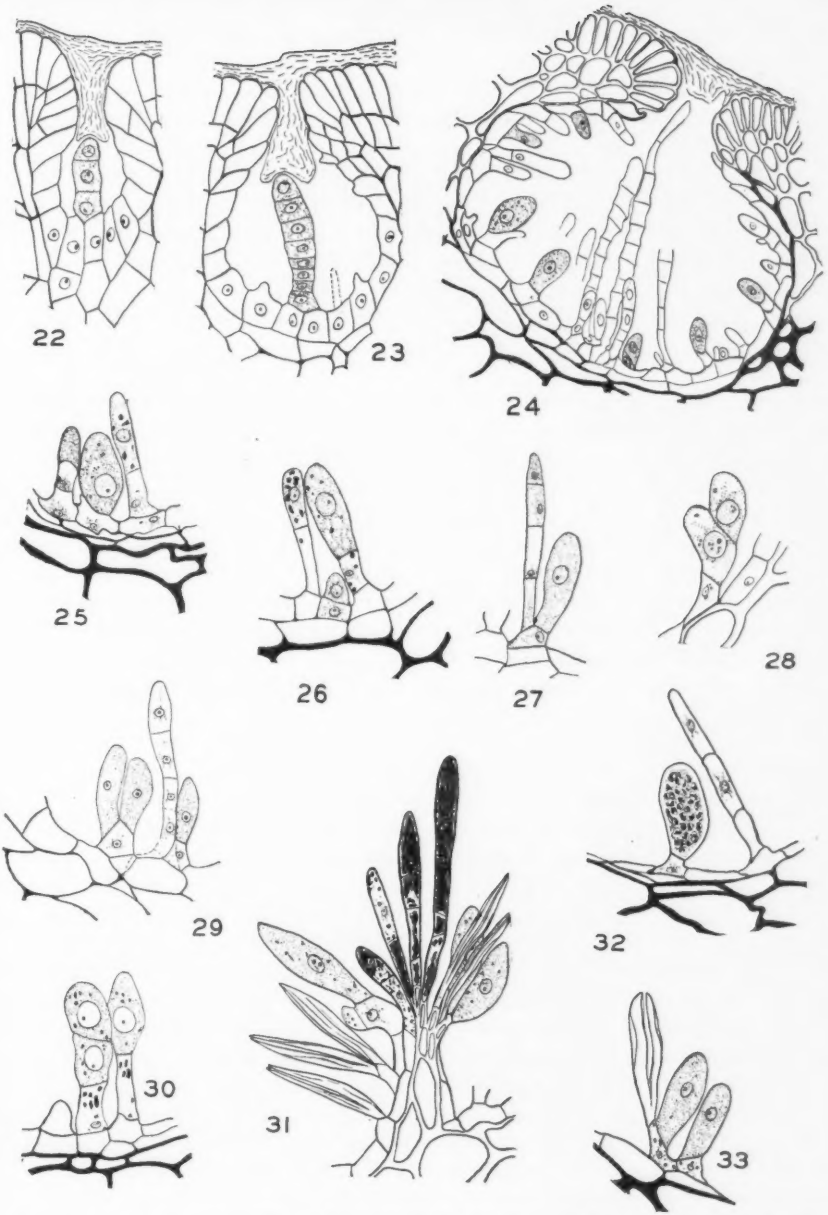
mal paraphysis, which, owing to its early start, may be distinguished for some time from the surrounding paraphyses by its greater length (figs. 22–24). In oogonial conceptacles, however, it usually disintegrates before it divides, or after it has divided into two or three cells, and gives rise to considerable mucilaginous substance (figs. 12, 14–16). By this difference in the fate of the tongue cell it is possible to distinguish the antheridial from the oogonial conceptacles in the early stages of development. Hermaphroditic conceptacles resemble the antheridial in that the tongue cell commonly develops into a paraphysis.

The inner of the two cells formed in the first division of the conceptacle initial has been variously called: the "inner cell" (23), the "basal cell" (18), the "conceptacle initial" (6), and the "true conceptacle initial" (3). To avoid confusion it seems best to designate the original cell as the conceptacle initial and the inner of the two cells resulting from its first division as the inner or basal cell. In *T. turbinata*, as in *Sargassum* and all the other genera so far investigated, division of the basal cell originates the development of the cells lining the conceptacle. This development in *T. turbinata* agrees with that of *Sargassum* as shown by the work of SIMONS and NIENBURG (19), as well as with that of most other genera in which it has been studied. The first division is by a median longitudinal wall (figs. 7, 10), which is followed in the resulting cells by similar divisions in the two planes until about sixteen cells are formed (figs. 8, 9, 11–13). A cross-section at this stage shows that division is of a quadrate type, with no indication that any one cell acts as an initial (fig. 13). This stage is usually followed by a transverse division of the peripheral cells, which are now slightly raised above the inner, owing to the concavity of the floor of the young conceptacle (figs. 9, 12). By repeated longitudinal division of the inner cells and by one in the peripheral, followed by some transverse divisions, the lining of the conceptacle is finally formed (figs. 14–17).

PARAPHYSES

In all conceptacles the central area of the floor is sterile, producing a group of paraphyses (fig. 24). This feature was first observed by SAUVAGEAU (22) in *Cystoseira* and has since been noted in *Carpophyllum* (7, 3). DAWSON (4) suggested that this feature may be related to the proliferation of branches which originate from within the conceptacles in *Notheia anomala* Bail. and Harv. (9, 30) and in an anomalous form of *Fucus ceranoides* L. (24). Some of these hairs extend to the ostiole or beyond. Paraphyses also originate in the fertile region of the conceptacle, between the antheridia and oogonia, but these are shorter than the basal hairs and decrease in length upward.

The origin of paraphyses in *T. turbinata* is similar to that of other members of the Sargassaceae. A cell in the lining of the conceptacle divides periclinally. The outer of the resulting cells develops a papilla in its free wall (fig. 20). The nucleus then divides and one of the daughter nuclei migrates into the papilla, which becomes separated from its mother cell by a wall commonly oblique to the outer surface (figs. 18, 19). This papilla then enlarges and by trichothallic growth develops into a paraphysis. Occasionally two papillae develop next to each other in adjacent cells, and therefore may appear to have originated by forking (fig. 18). No truly forked hairs have been observed in *T. turbinata* such as were noted by DAWSON (3) in *Carpophyllum*



FIGS. 22–33.—Fig. 22, young antheridial conceptacle showing three-celled tongue cell paraphysis. Fig. 23, antheridial conceptacle showing eight-celled tongue cell paraphysis. Fig. 24, nearly mature antheridial conceptacle showing group of paraphyses in center of floor of conceptacle. Figs. 25–30, young antheridia. Fig. 31, proliferation with tuft of antheridial filaments. Fig. 32, mature antheridium. Fig. 33, young antheridium developing below older empty one.

flexuosum. Branching of paraphyses does not occur except in the short hairs ("peripheses") below the rim of the ostiole (fig. 21). These hairs may be related to antheridial filaments.

ANTHERIDIA

The development of antheridia, or "spermatocysts," among the Sargassaceae has been studied in the following genera: *Sargassum* (**23, 16**), *Coccophora* (**27**), *Bifurcaria* (**21**), and *Cystoseira* (**4**). The accounts of KUNEIDA and TAHARA are the most comprehensive, as they extended their studies to include the cytological features.

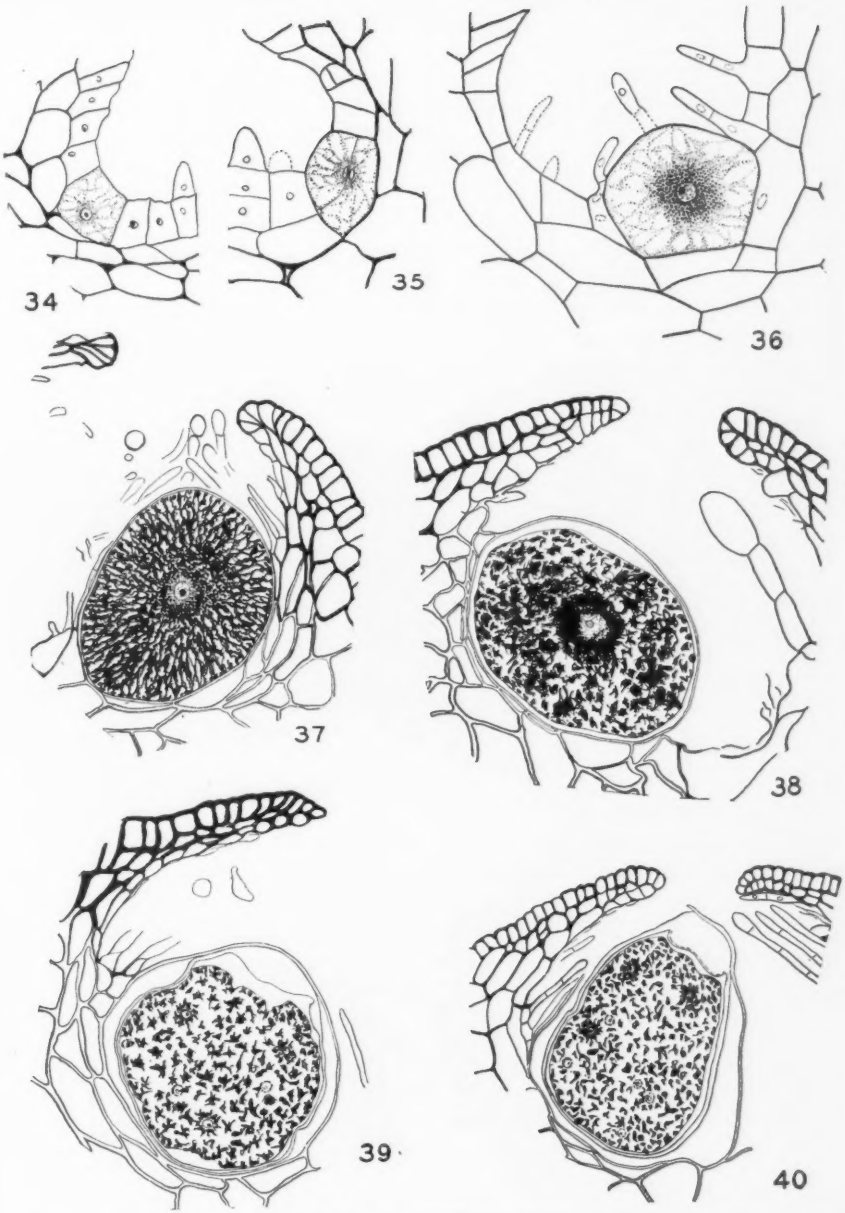
The morphological features in *T. turbinata* conform in general with those of the other Sargassaceae; no attempt has been made to study its cytology. The origin of antheridial filaments is the same as that of paraphyses. In fact, at first the two are indistinguishable. Antheridial cells are first distinguishable from sterile cells by their denser cytoplasm and larger nuclei (fig. 25). In their position they show considerable variation. They may be essentially sessile (fig. 25) as in *Sargassum filipendula* (**23**) and *Cystoseira foeniculacea* (L.) Grev. (**4**), or stalked (fig. 30) as in *Sargassum horneri* (Turn.) C. Ag. (**16**), or branches of filaments (fig. 27). Occasionally, antheridia are formed in short rows (figs. 28, 30) as in *Coccophora langsdorfii* (Turn.) Grev. (**27**). In stalked forms the filament is usually simple at first, but the stalk may later give rise to an antheridium by branching (figs. 29, 31, 33). Thus younger antheridia often appear below the older ones (fig. 33). Rarely, proliferating outgrowths appear that bear a tuft of antheridial filaments (fig. 31). Commonly, a conceptacle simultaneously has antheridia in different stages of development. No proliferation of antheridia up through the older empty ones has been observed, as was noted by DAWSON (**4**) in *Cystoseira foeniculacea*. In hermaphroditic conceptacles the antheridia appear above the oogonia (protrandrous), as has been reported for other forms.

The sperms are discharged en masse, and apparently this occurs periodically, coinciding with the period of liberation of oogonia, as was observed by ABE (**1**) in *Sargassum horneri*.

OOGONIA

The cells from which the oogonia or "oocysts" are destined to be formed are located on the lower side of the conceptacle and are derived from the peripheral cells of the lining. They are first distinguishable from the surrounding cells by their larger size and larger nuclei (fig. 34). This position of the oogonial mother cells in *T. turbinata* corresponds with that of other genera investigated. Their arrangement in relation to one another, however, does not agree with that of *Carpophyllum* as reported by DELF (**7**) and by DAWSON (**3**). In this genus the arrangement is reported to be spiral, and oogonia are therefore of different ages within one conceptacle. The explanation of this arrangement rests upon the assumption that "the 'conceptacle initial' behaves as a specialized apical initial" (**3**). In *T. turbinata* there is no indication that the oogonia are spirally arranged or differ markedly in age within one conceptacle—as in *Sargassum* and *Cystophyllum* (**25, 26**). Furthermore, a transverse section of the early products of the basal cell shows no indication that any initial cell is maintained (figs. 10, 13).

The oogonial initial cell divides periclinally into two cells, the outer of which becomes the oogonium mother cell without further division (fig. 35). The inner



FIGS. 34–40.—Fig. 34, oogonial initial cell. Fig. 35, division of oogonial initial forming oogonial mother cell and stalk cell. Fig. 36, young oogonium. Fig. 37, oogonium just before formation of mesochiton. Fig. 38, oogonium with developing mesochiton. Fig. 39, eight-nucleate oogonium with mesochitonous pad. Fig. 40, eight-nucleate oogonium inside ruptured exochiton.

or "stalk" cell does not function as a stalk in raising the oogonium above the surrounding cells but is soon crushed by the rapidly enlarging oogonium (fig. 37).

During the early development of the oogonium there is no indication that hairs develop on its outer wall, as DAWSON (4) observed in *Cystoseira foeniculacea*. However, as the oogonium expands into the surrounding cells, the paraphyses located near the oogonium come to lie on its outer surface (figs. 36, 37).

The first indication of differentiation of the oogonium from the oogonium mother cell is the appearance of a number of small chromatophores around its nucleus (fig. 36). As the oogonium enlarges these plastids increase in number, size, and extent, eventually becoming irregular in shape and somewhat radiating from the center (fig. 37). The enlarging oogonia exert considerable pressure on the surrounding cells, which become distorted into plates and columns between the oogonia. Owing to the elliptical shape of most of the conceptacles (brought about by elongation of the receptacles), the oogonia are more crowded transversely than longitudinally. Oogonia which have reached their final stage of development before liberation vary somewhat in shape but are commonly elliptical. Their average size is $140 \times 174\mu$. Each oogonium possesses a large nucleus with granular chromatin, which is characteristic of the Sargassaceae. Three to six oogonia are borne in each conceptacle, but five is the most common number.

NUCLEAR MATURATION AND DISCHARGE

The first indication that an oogonium has approached the stage of liberation is the production of a thick mucilaginous layer between the original cell wall and the protoplast (fig. 38). This layer has been called "mesochiton," while the original cell wall has been termed the "exochiton." No "endochiton" has been observed in *T. turbinata*, as has been reported in *Cystophyllum flexuosum* (3) and *Cystoseira foeniculacea* (4). In the outer region of the oogonium, the mesochiton thickens more rapidly than elsewhere and forms a distinct cushion or pad (figs. 38, 39). This pad was first observed by KUNEIDA (16) in *Sargassum horneri*. It is no doubt largely responsible for the rupture of the exochiton on one side at the upper end, forming a lid which is hinged on the opposite side (fig. 40). In *T. turbinata* this mesochitonous pad does not seem to slide down the side of the oogonium, as has been observed in *Sargassum horneri* (16), *Carpophyllum flexuosum* (3), and *Cystoseira foeniculacea* (4). The subsequent events leading to the discharge of the oogonium have not been observed, but from the final orientation of the oogonium and the basal attachment of the mucilaginous stalk which holds it they may be visualized as follows. In emerging from the exochiton, owing to the attachment of the mesochitonous pad to the lid of the exochiton, the oogonium is inverted, so that the basal end becomes the distal end in the discharged oogonium (fig. 41). Accompanying this emergence, the mesochitonous pad becomes attenuated into a strand which remains attached to the lid of the exochiton within the conceptacle, thus anchoring the discharged oogonium (fig. 41).

The discharge of oogonia is an interesting feature which was first observed by TAHARA (25) in *Sargassum enerve* C. Ag. He found that the discharge was periodic and simultaneous in all plants and occurred at fortnightly intervals that coincided with the spring tides. The oogonia are discharged in zones, commencing at the base of the

receptacle and progressing upward for each succeeding period. This periodicity was investigated further by INOH (**11**) in twelve species of *Sargassum*, and he found that all discharge oogonia periodically in relation to the spring tides, although they may fruit at different seasons.[1] Discharge usually takes place 1–3 days after the highest tide. TAHARA (**26**) found that, in artificial cultures, discharge of oogonia was considerably delayed. He also concluded that in *Sargassum thunbergii* O'Kuntze, discharge of oogonia probably occurs at neap tide, which—if true—represents an exception to all other species in which periodicity has been noted.

Although apparently no special studies have been made on periodicity of oogonial discharge in other genera, it has been noted in *Cystophyllum* (**26, 12–14**) and in *Turbinaria fusiformis* (**28**), and indications are that it is also present in *Carpophyllum* (**3**) and *Bifurcaria* (**6**). While this phenomenon is no doubt widespread among the genera of the Sargassaceae, and evidently also in some of the Fucaceae, it must not be concluded that it is universal. TAHARA (**27**) found in *Coccophora langsdorfii* that, while discharge was simultaneous in all conceptacles, it was not periodic. In *Cystoseira foeniculacea*, DAWSON (**4**) found evidence of periodicity inconclusive, although indications are that it is present in this species also.

While no special effort was made to determine periodicity in *T. turbinata*, from the fact that the oogonia are discharged in definite successive zones (fig. 2) it may be concluded that the discharge is periodic in this alga also. From examination of receptacles of different ages, it appears that oogonia are discharged in three zones (fig. 2), as has been observed in other forms. Generally, all oogonia are discharged from each conceptacle at one period. Occasionally, however, one may remain behind, owing to some interference in the process or some lack of maturity. That the latter is probably the more common reason is indicated by the fact that this irregularity is most frequent in the upper (younger) margin of the discharged zone.

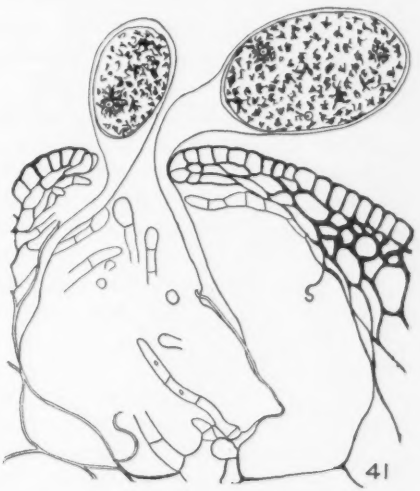


FIG. 41.—Oogonial conceptacle with discharged, attached eight-nucleate oogonia.

The cause or causes of this periodicity in the discharge of oogonia in the Sargassaceae are not definitely known. It has been suggested (**6**), however, that light and tide variation may be important factors.

Another interesting feature associated with the discharge of oogonia is their attachment. DODEL-PORT (**5**) first observed that oogonia adhere on the outside of the receptacle in *Cystoseira barbata*. In this species they are simply held

[1] Observations on *Sargassum filipendula*, the common and widespread species of the Atlantic coast of North America, indicate that this species also discharges oogonia at fortnightly periods at the time of spring tides.

by mucilage to cryptostomatal hairs. A similar situation was found by INOH (14) in *Cystophyllum crassipes* (Mert.) J. Ag. and by OKABE (20) in *C. sisymbrioides* (Turn.) J. Ag. In the majority of instances, however, they are held by a mucilaginous stalk which originates from the mesochiton. While oogonia thus attached had been observed by SIMONS (23) and TAHARA (25), the first one to have actually noted the stalks was NIENBURG (18). The species in which this structure has been observed since that time have been tabulated by DAWSON (4).

Oogonia are included within their individual mesochitonous sheaths during the period of attachment and are in addition collectively imbedded in a copious, transparent, mucilaginous substance (fig. 2). The origin of this substance has not been determined in *T. turbinata*, but it probably comes largely from the paraphyses, an opinion held also by other investigators.

As already stated, NIENBURG (19) was the first to conclude that the nucleus of the oogonium undergoes meiosis, although he did not actually observe the process. Apparently, owing to the rapidity with which it occurs, it is difficult to obtain cytological evidence. TAHARA (29) seems to have been the first and only one who has succeeded in observing meiosis in the oogonium in the Sargassaceae—in his work on the genus *Coccophora*. From circumstantial evidence, however, it is generally assumed that meiosis does take place, and this applies also to the present work on *Turbinaria*. To obtain mitotic or meiotic figures in the Phaeophyceae necessitates special effort in collecting and fixation, which it is hoped can be undertaken at some future time in *Turbinaria*.

In *T. turbinata*, as in the majority of the members of the Sargassaceae which have been studied, reduction division is followed by another division, resulting in eight nuclei. Exceptions are *Coccophora langsdorfii* (28) and *Bifurcaria laevigata* (Kütz.) Delf and Mitch. (17), which evidently have only four.

As to the location of the oogonium at the time of meiosis—that is, whether it takes place before or after discharge—there seems to be considerable variation in the different genera. This may be more apparent than real, however, because of the difficulty of observing oogonia immediately prior to or during the process of discharge. There is also the possibility that it takes place both before and after discharge in the same plant.

In *T. turbinata* reduction division seems to occur prior to discharge, as a few conceptacles were found which showed oogonia *in situ* with eight nuclei (figs. 39, 40). Of course, the possibility exists that these oogonia were for some reason delayed in being discharged, and therefore represent an abnormality. However, eight-nucleate oogonia within the conceptacle were observed by KUNEIDA (16) in *Sargassum horneri;* and from reports by other observers on other genera it may be concluded that in the majority of forms meiosis takes place in the oogonium prior to discharge. A definite exception is presented by *Coccophora*, however, in which TAHARA (28) observed only a single nucleus in the oogonium at time of discharge.

It has not been possible to determine definitely the method of elimination of the seven supernumerary nuclei from the egg, but—from the stages available—indications are that they disintegrate within the cytoplasm, as occurs in *Sargassum* and in the other species of *Turbinaria* which have been studied.

Embryogeny

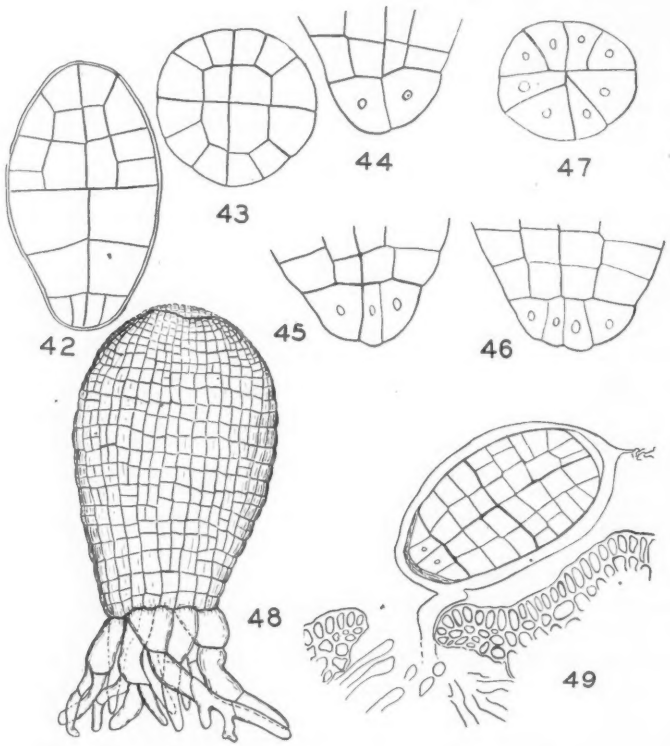
The first observations on embryological development in the Sargassaceae were made by Simons on *Sargassum filipendula*. She noted that the fertilized egg remains attached to the outside of the receptacle until a several-celled structure has been formed, and that "The first division of the egg in *Sargassum* does not differentiate a rhizoidal region, as in *Fucus* and *Ascophyllum*." She also observed that several rhizoids develop at one end after the embryo has grown into many cells. Nienburg (**18**) found in *Sargassum linifolium* that the first wall of the fertilized egg is laid down across the middle and that a later division cuts off at one end a lenticular rhizoidal cell. Subsequent work on the embryogeny of the Sargassaceae has been done mainly by Japanese investigators, who have paid special attention to early segmentation and rhizoidal formation.

In summarizing studies on the embryogeny of the Sargassaceae, the following facts seem to have been pretty well established:

1. In all forms investigated, the developing embryo usually remains attached to the outside of the receptacle until it has developed into a several-celled structure. This change requires a period of 2–3 days.
2. The fertilized egg is first divided into approximately two equal cells across the shorter diameter if the egg is ellipsoidal.
3. At the second division, a small lenticular cell is cut off at one end, although *Sargassum linifolium* may be an exception, for—according to Nienburg (**19**)—this occurs at the third division.
4. The embryo proper develops by a regular quadrate method of cell division until it has become many-celled and an apical cell is established.
5. There is considerable variation among the different genera—and even among the species of one genus (*Sargassum*, **11**)—in the number of rhizoids and their formation.
6. Primary rhizoidal cells are formed by longitudinal divisions of the rhizoidal cell. In a few forms, however, a "two-storied" development may result from transverse divisions of the first cells formed (*Cystophyllum*, **26, 20**; *Sargassum patens*, **11**).
7. The number of primary rhizoids is usually four, eight, sixteen, or thirty-two, with some individual variation.
8. Secondary rhizoids may arise from the basal cells of the embryo proper or from the inner primary rhizoidal cells.
9. The number of primary rhizoids and their method of formation are apparently of no great systematic significance.

In the material of *T. turbinata* used in this study a few embryological stages have been obtained, but unfortunately these do not include the early ones. However, from the stages available (figs. 42, 43) it is apparent that development conforms in general with that of other genera and other species of *Turbinaria* (**28, 13**). The lenticular rhizoidal cell is divided radially by two perpendicular walls into four approximately equal cells (fig. 44), each of which is divided once by oblique walls (figs. 42, 45, 46), resulting in eight primary rhizoidal cells from which grow eight primary rhizoids. This agrees with the development and number of rhizoids in several species of *Sargassum* (**28, 11**), and in *Coccophora* (**27**), *Turbinaria fusiforme* (**28**), *T. ornata* and

T. filiformis (14). A few advanced embryos were encountered with well-developed primary rhizoids (fig. 48). These are unique in two respects: first, they show forking which has been seen only in *Cystophyllum crassipes* (14); second, they indicate branching, which has not been reported previously. No stages have been reached. From examination of sections of a number of embryos of *T. turbinata*, it appears that the rhizoidal end is commonly directed toward the ostiole (fig. 49). This would indicate that the rhizoidal end is the original outer end of the oogonium which becomes inverted in the process of emergence.



FIGS. 42–49.—Fig. 42, longisection of young embryo. Fig. 43, transection of young embryo. Figs. 44–46, longisection of rhizoidal cell showing divisions. Fig. 47, transection of rhizoidal cell showing eight primary rhizoidal cells. Fig. 48, many-celled embryo with primary rhizoids. Fig. 49, longisection of embryo showing orientation.

were observed sufficiently advanced to show secondary rhizoids.

In the study of embryogeny in this family there has been some speculation concerning the orientation of the embryo, but apparently no definite conclusions

In conclusion, it might be said that, in its reproductive structures and their development, *T. turbinata* appears to conform closely with that of the majority of genera and species of the family Sargassaceae so far investigated.

Summary

1. *Turbinaria turbinata* occurs in Puerto Rico, mostly on the northern or Atlantic side, on rocky substrata in shallow depressions or to a depth of 5 feet.

2. Plants are commonly of one sex, but not infrequently hermaphroditic as well as antheridial and oogonial conceptacles appear in the same receptacle.

3. Receptacles originate from a triangular apical cell which in longitudinal sections appears lenticular.

4. In origin and development the conceptacles are like those of *Sargassum* and most of the other genera and species of the Sargassaceae which have been studied.

5. In the male conceptacle a normal paraphysis develops from the tongue cell, but in the oogonial conceptacles it disintegrates early and gives rise to a mucilaginous substance.

6. The central area of the floor of the conceptacle is sterile and bears a tuft of unbranched paraphyses, as has been observed in a few other genera.

7. The antheridia vary in position from being essentially sessile to being stalked or as branches of filaments. Occasionally they occur in short rows, as in *Coccophora*.

8. Oogonia develop in the lower region of the sides of the conceptacle and are approximately of the same age within each conceptacle. Five is the most common number.

9. Eight-nucleate oogonia have been seen *in situ*, indicating that meiosis occurs just before or during emergence.

10. The oogonium develops a mesochiton which in the outer region forms a pad. This pad ruptures the exochiton and elongates into a stalk which anchors the discharged oogonium.

11. The embryo has eight primary rhizoids which are commonly situated at the end proximal to the ostiole.

12. Except for a few minor differences, *T. turbinata* conforms in its reproductive structures to that of *Sargassum* and the majority of other Sargassaceae.

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MEIOSIS IN AUTOTETRAPLOID LOLIUM PERENNE IN RELATION TO CHROMOSOMAL BEHAVIOR IN AUTOPOLYPLOIDS[1]

W. M. MYERS[2]

Introduction

The irregularities of meiosis and, consequently, the low fertility of autopolyploids have been attributed by DARLINGTON (**7**) to the multivalent association of chromosomes during synapsis. This hypothesis has been supported by several investigators, particularly KOSTOFF (**14, 15**). It is evident from the literature that there has been a general tendency to accept multivalent frequency as a criterion of the relative meiotic irregularity of various autopolyploids. KOSTOFF (**15**) concluded that, in species with short chromosomes, the autopolyploids would tend to be more regular in meiosis because of lower chiasma frequency and, hence, fewer multivalents. This hypothesis was adopted by EARN-

[1] Contribution no. 69, of the U.S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, State College, Pennsylvania, in cooperation with the northeastern states.

[2] Geneticist.

SHAW (9) to account for the fertility of tetraploid sea plantains allied to *Plantago maritima* L. From studies of meiosis in tetraploids and in hybrid triploids, he concluded that the 4x plants were autotetraploids. The quadrivalent frequency was low, never more than two per cell, a fact that he attributed to the low chiasma frequency.

In *Dactylis glomerata* L., which behaves cytogenetically like an autotetraploid (x = 7), MYERS (30) and MYERS and HILL (35) found, however, that variation in quadrivalent frequency was not a reliable measure of the irregularity of meiosis. Unequal disjunction of the quadrivalents at anaphase I occurred only infrequently in this species. On the other hand, unpaired chromosomes at metaphase I were found in considerable frequency in most plants. Because of their tendency to lag and divide equationally at anaphase I and to be left in the cytoplasm at telophase I and II, these unpaired chromosomes were an important contributing factor to the formation of aneuploid gametes. The frequency of unpaired chromosomes was not significantly correlated with quadrivalent frequency among unrelated plants of *Dactylis* (34, 30). Quadrivalent frequency was positively correlated with chiasma frequency, which in turn was negatively correlated with incidence of univalents at metaphase I. It was concluded, therefore, that reduction in quadrivalent frequency resulting from reduced numbers of chiasmata would be accompanied by an increase in univalents at metaphase I (30). Hence, plants with low chiasma frequency would be on the average less regular in meiosis than those with high frequency.

Data from induced autotetraploids of *Lolium perenne* L., reported in this paper, provide further information regarding irregularities of meiosis in autopolyploids. These data are of special interest for comparison with meiotic behavior in naturally occurring autotetraploid grasses, such as *Dactylis glomerata*.

MATERIAL AND METHODS.—The material consisted of pairs of diploid and tetraploid clones obtained by HILL and MYERS (12) from chimeral plants produced by treatment of germinating seeds of *Lolium perenne* with solutions of colchicine (25). In this report, the related diploid and tetraploid clones are identified by the number of the original seedling, followed by 2x and 4x, respectively, as—for example—C50–2x and C50–4x. Microsporocyte material was collected from twelve pairs of diploid and tetraploid clones growing in adjacent rows in the field. Later, material was collected in the greenhouse from five pairs of clones, including three of those grown in the field and two additional ones.

The microsporocyte material was killed and fixed in acetic alcohol and stored in the fixing solution in a household refrigerator. All data were collected and photomicrographs were made from fresh aceto-carmine smear slides.

Experimental results

TIMING BALANCE IN MEIOSIS

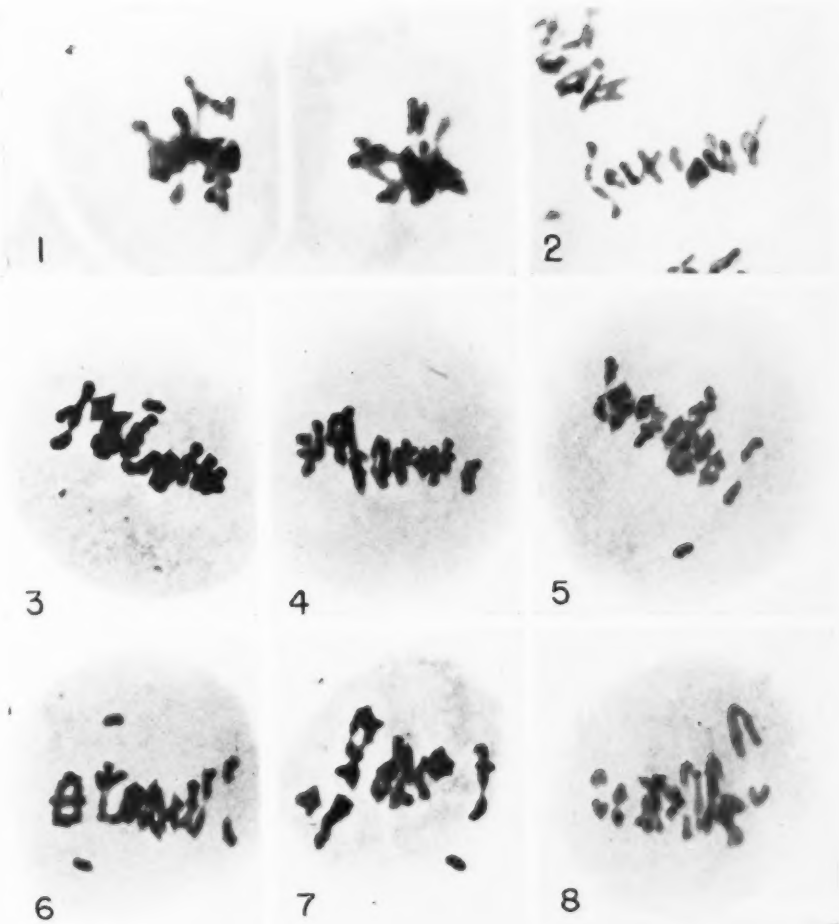
In contrast to most diploid plants of *Lolium perenne* and to normal plants of other grass species that have been examined by the writer, well-spread diakinesis preparations were never obtained from the autotetraploid plants of *L. perenne*. Contraction of the chromosomes, attributed to despiralization by SWANSON (44), was delayed relative to the progression of the stages of meiosis. The chromosomes were still long and

tangled when congression on the equatorial plate commenced. In most plants, contraction of the chromosomes continued and apparently was completed at metaphase I, prior to the initiation of anaphase I. In these plants, well-spread and readily interpreted metaphase I sporocytes were obtained (figs. 2–8). In one clone (C108–4x), however, contraction was not completed and the chromosomes did not become oriented in an orderly manner at metaphase I (fig. 1). In this clone, interpretation of metaphase I was extremely difficult. This difference in synchronization of chromosome contraction, congression on the equatorial plate, and separation at anaphase I was not observed in C108–2x compared with other diploid clones. Behavior similar to that in C108–4x was observed in C190–4x. In this clone orientation was so disorderly and the chromosomes so tangled that interpretation of pairing was not possible in any sporocyte at metaphase I.

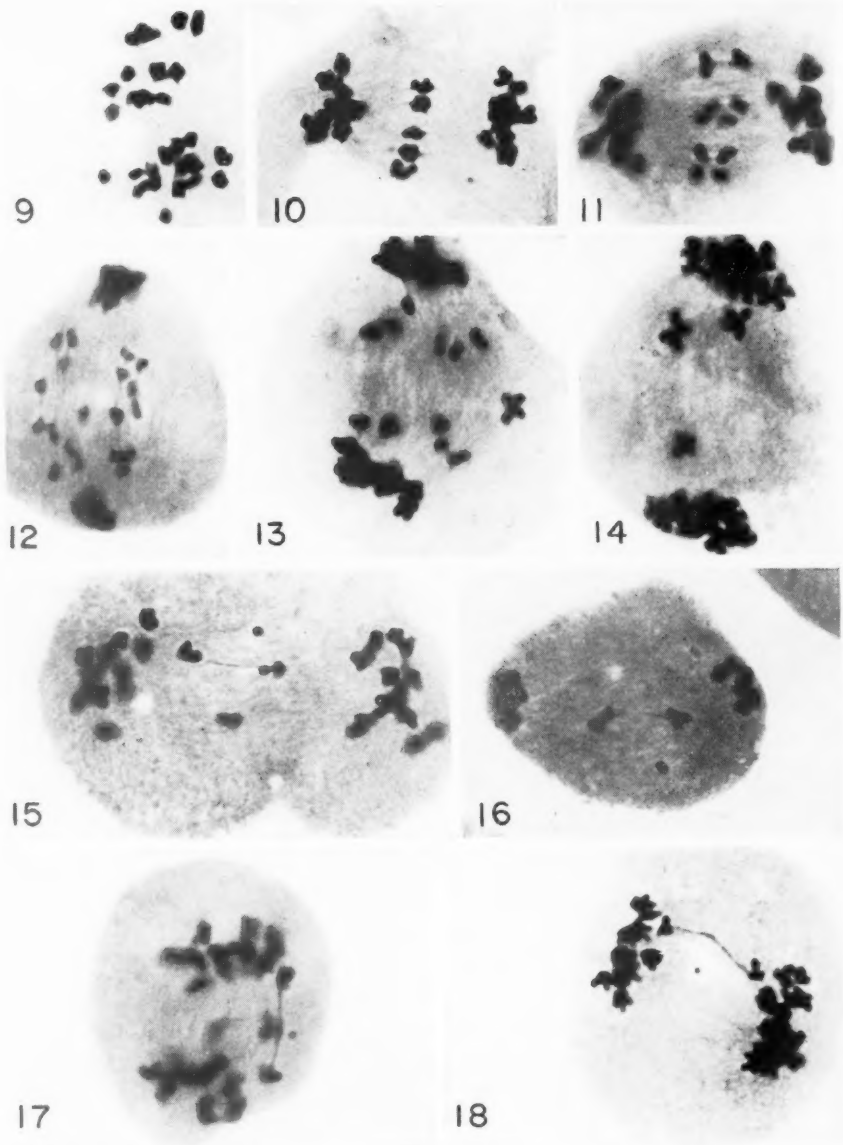
The clone C108–4x differed from other clones also in the progression of meiotic stages among florets of the same spikelet and among spikelets of the same inflorescence. In *L. perenne* the lowermost spikelet is the youngest and the stage of development of the microsporocytes is progressively more advanced from spikelet to spikelet as the top of the spike is approached. Within the spikelet the basal floret is the most advanced in development and the terminal floret the least. In each pair, except C108, the rate of progression (development of the microsporocytes) between adjacent florets and adjacent spikelets in the tetraploid was indistinguishable from that in the diploid. In C108, progression was noticeably slower in the tetraploid than in the diploid. This appears to provide further evidence of the upset of the orderly process of meiosis resulting from chromosomal reduplication in this clone.

Chiasma frequencies of diploid and tetraploid clones

Upcott (46) concluded that the chiasma frequency per chromosome might be lower in polyploids than in comparable diploids, owing to delay of pairing resulting from the larger nucleus of the polyploid. Evidence was obtained from autotetraploid *Primula kewensis* that supported this hypothesis. Furthermore, Upcott (46) cited nine reports from the literature in which the chiasma frequency of the polyploid was lower than that of the comparable diploid. Similar results were obtained by Richardson (41) in *Setcreasea brevifolia*, Anderson and Sax (1) in *Tradescantia*, Kostoff (16) in *Petunia hybrida*, and Peto (37) in induced autotetraploid *Hordeum vulgare*. Contrary results were obtained by Levan (19) in the *Allium paniculatum* group. Earnshaw (9), in studies of sea plantains allied to *Plantago maritima*, obtained data on chiasma frequencies from five diploid and four tetraploid plants; on the average there was little difference between them. In *Dactylis*, Müntzing (23) reported that the chiasma frequency per chromosome in the diploid *D. aschersoniana* was about as high or only slightly lower than in the tetraploid *D. glomerata*. The validity of most of these comparisons is impaired by one or both of two factors: (*a*) data were collected from too few sporocytes, and (*b*) the polyploids were not genetically comparable with the diploids. In five of the nine cases cited by Upcott (46), chiasma frequency of the polyploid was determined from one or two cells. The inadequacy of such data is evident when the variation in chiasma frequency



FIGS. 1–8.—Fig. 1, metaphase I with irregular orientation. Figs. 2, 3, 4, types of quadrivalents at metaphase I. Fig. 5, trivalent and univalent at metaphase I. Fig. 6, types of quadrivalents at metaphase I; three chromosomes of one quadrivalent oriented toward one pole. Fig. 7, univalent and trivalent at metaphase I. Fig. 8, disjunction of quadrivalent with three chromosomes moving to one pole.



FIGS. 9–18.—FIG. 9, anaphase I showing 13–15 distribution of chromosomes. Fig. 10, five lagging univalents at anaphase I showing equational split. Fig. 11, five univalents dividing at anaphase I. Fig. 12, daughter chromosomes from lagging univalents at telophase I. Fig. 13, daughter chromosomes from lagging univalents and one laggard with centromere not divided. Fig. 14, undivided laggards at interphase I. Fig. 15, short dicentric bridge and large acentric fragment in tetraploid clone. Fig. 16, dicentric bridge and acentric fragment in diploid clone from same pair as tetraploid clone of fig. 15. Fig. 17, dicentric bridge and acentric fragment of intermediate size. Fig. 18, long dicentric bridge and small acentric fragment.

among sporocytes of the same anther is considered. The importance of heritable differences in chiasma frequency among plants of the same species has been emphasized by MYERS (30) and MYERS and HILL (35). The necessity for controlling genetic differences was recognized also by KOSTOFF (16), whose data were obtained from diploid and tetraploid branches of the same plant, and by PETO (37), whose data were obtained from diploid and tetraploid florets of the same spike.

The clones used in these investigations provide material for a critical study of the effects of chromosomal reduplication on chiasma frequency, since the tetraploid clones should differ cytogenetically from their related diploid clones only in number of alleles of each gene and amount of chromatin material in the nucleus. For determination of chiasma frequencies, an average of twenty complete metaphase I sporocytes was analyzed for each tetraploid clone and an average of twenty-six sporocytes for each diploid clone. It was evident from these data (table 1) that the effect of chromosomal reduplication on chiasma frequency was not constant for all clones. The chiasma frequency of the field-grown tetraploid clones varied from 76 to 103% of the frequency of the respective diploid clones. In the absence of replication, the significance of these differences cannot be determined directly. In studies of variations in chiasma frequencies using replicated clones of *Dactylis glomerata*, MYERS (30) found that $2 \times$ S.E. of the difference for a single determination = 0.08. Using this value as a guide in comparing the results obtained in the present investigation, it is evident that in the field material the diploid and tetraploid clones of five pairs did not differ in chiasma frequency. In each of six pairs the tetraploid clone had a lower chiasma frequency than the diploid, and the differences probably were significant. The greatest reduction was in C108, which—as indicated earlier—showed evidence of an upset in timing balance in meiosis as a result of chromosome doubling.

Data from the greenhouse material were generally in agreement with those obtained from the field material. The differences were small and probably not significant in four pairs, while in the fifth pair the tetraploid may have been significantly lower than the diploid in chiasma frequency. Three of the pairs in which no difference was obtained were included in the field material, where the differences likewise were not significant. The correlation coefficient of chiasma frequency of the tetraploid clones with that of the diploids was 0.316 (14 D/F), a nonsignificant value.

In contrast to *D. glomerata*, in which almost all the chiasmata are completely terminalized, both terminal and subterminal chiasmata occur in diploid *L. perenne* (26). Subterminal chiasmata were found also in the induced autotetraploids (figs. 3–7). Quantitative data on degree of terminalization were not collected in these investigations, but the tetraploid clones did not differ noticeably from the diploids in this regard.

QUADRIVALENTS AT METAPHASE I

In average quadrivalent frequency per sporocyte, the tetraploid clones varied from 2.92 to 4.83, with an average for all clones (including two determinations from each of three of the clones) of 3.96 (table 1). The range in number of quadrivalents is similar to values obtained for unrelated plants of *D. glomerata* by MYERS and HILL (34, 35) and MYERS (30).

DARLINGTON (**6, 7**) has diagramed the ten types of quadrivalents possible in an autotetraploid. Six of these types (DARLINGTON'S numbers 11, 12, 13, 15, 16, and 17) were found in this material. Among the 1252 quadrivalents recorded, the most frequent types were those with symmetrical arrangements, that is: the simple ring, type 17 (61.8%); and simple chain, type 11 (29.4%). These types are shown in figures 2, 4, 5, 6, and 7. Type 12 (fig. 3) was observed twelve times (0.9%); type 13 (figs. 2, 6) was observed sixteen times (1.3%); type 15 was found seventeen times; and type 16 occurred sixty-four times (5.1%). A preponderance of symmetrical types is expected in a species with a chiasma frequency such as found in *L. perenne*, since only one chiasma per chromosome arm is required for their formation, whereas two chiasmata per chromosome arm (in at least two chromosomes) are required for formation of the other types

TABLE 1

COMPARATIVE DATA ON MEIOTIC BEHAVIOR IN PAIRS OF DIPLOID AND TETRAPLOID CLONES OF LOLIUM PERENNE; EACH PAIR DERIVED BY VEGETATIVE PROPAGATION FROM A SINGLE COLCHICINE-TREATED GERMINATING SEED

Clone no.	X-ta per chromosome			IV per sporocyte in 4x	MI with univalents (%)		AI with laggards (%)		Quartets with micronuclei (%)	
	2x	4x	Ratio		2x	4x	2x	4x	2x	4x
	Field-grown material									
101	1.80	1.75	97	4.06	1.7	30.6	2.2	44.6	2.1	56.6
102	1.66	1.71	103	3.26	0.9	17.3	0	31.4	2.0	41.8
108	1.96	1.49	76	3.67	1.3	51.8	0	55.6	0	54.0
116	1.89	1.83	97	4.31	1.9	28.8	0.9	26.6	2.8	25.0
166	1.82	1.65	91	4.05	1.3	38.1	1.4	59.3	0.5	62.8
190					37.9	45.5	54.6	95.2	58.5	95.8
198	1.77	1.76	99	4.44	1.2	22.1	1.7	45.4	6.4	54.5
214	1.63	1.36	83	2.92	1.8	26.2	0	17.4	1.2	53.0
218	1.74	1.74	100	3.57	1.8	24.8	1.9	17.3	1.4	40.3
241	1.87	1.74	93	4.83	1.3	23.9	2.3	45.0	0.8	27.1
250	2.05	1.81	88	3.30	0	19.1	0	26.4	0.5	14.6
251	1.94	1.80	93	3.71	1.3	19.2	1.0	25.0	2.7	49.1
Av.	1.83	1.69	93	3.83	4.4	29.0	5.5	40.8	6.6	47.9
	Greenhouse-grown material									
50	1.84	1.69	92	4.50	4.8	27.1	0.8	43.4	0.5	34.4
101	1.67	1.74	104	4.07	4.1	35.9	3.9	48.4	5.4	45.2
183	1.69	1.79	106	3.68	4.0	20.6	0.2	21.3	0.7	28.0
198	1.92	1.85	96	4.62	4.3	26.2	1.1	51.1	3.1	55.4
218	1.84	1.90	103	4.44	0.9	18.3	2.3	20.3	1.1	17.1
Av.	1.79	1.79	100	4.26	3.6	25.6	1.7	36.9	2.2	3.6
Grand av.	1.82	1.73	95	3.96	4.4	28.0	4.4	39.6	5.6	44.4

of quadrivalents. Quadrivalent frequency was significantly correlated with chiasma frequency in this material ($r = 0.517$, $P < 0.05$).

The unequal disjunction at anaphase I of the chromosomes of multivalents has been reported by DARLINGTON (**7**) to be the principal cause of instability and low fertility in autopolyploids. In this material unequal disjunction apparently occurred frequently. At metaphase I quadrivalents were found in which the members were oriented in a manner to suggest the probability that three chromosomes would pass to one pole and one chromosome to the other at anaphase I. In figure 6 the second quadrivalent from the left is oriented in this manner. At early anaphase I, quadrivalents were observed in which the separation of three chromosomes to one pole was evident (fig. 8, quadrivalent on extreme right). This unequal disjunction of members of quadrivalents would be expected to result in the production of aneuploid daughter nuclei at telophase I, and hence of aneuploid gametes. At anaphase I, the number of chromosomes in both groups could be determined with certainty in forty sporocytes in which no lagging occurred. There was a 14–14 distribution in twenty-one (52.5%), 13–15 in sixteen (40%), and 12–16 in three (7.5%). A sporocyte with a 13–15 distribution is shown in figure 9.

OCCURRENCE AND BEHAVIOR OF UNIVALENTS AT METAPHASE I

The incidence of univalents was determined from an average of 249 metaphase I sporocytes in the diploid clones (table 1). The frequency was low in all clones except C190–2x, being similar to that reported previously for diploid plants of this species (**26**). In C190–2x, 37.9% of the metaphase I sporocytes had two (or occasionally four) unpaired chromosomes.

The frequency of sporocytes with univalents in the tetraploid clones, determined from an average of 224 metaphase I sporocytes, was considerably higher than in the diploids, varying from 17.3 to 51.8%. In some instances there was a single univalent plus a trivalent (figs. 5, 7), but more commonly two or more univalents were found (fig. 6)—the incidence of trivalents being relatively low in this material.

The frequency of univalents in C190–4x (45.5%) was lower than expected from the behavior of C190–2x. Since metaphase I was extremely difficult to analyze in this clone, it is probable that some univalents were overlooked and that this value is too low. This assumption is supported by the high incidence of lagging at anaphase I (95.2% of the sporocytes with laggards).

Variations among tetraploid clones in incidence of asynapsis at metaphase I may be attributed in part to variations in chiasma frequency. The coefficient of correlation between these characters was -0.516 ($P < 0.05$). On the other hand, metaphase I univalent frequency was not correlated with number of quadrivalents ($r = 0.048$). Also, metaphase I univalent frequency of the tetraploid clones was not significantly correlated with metaphase I univalents of the diploids ($r = 0.255$, $P < 0.10$) when data from C190 were omitted in the calculation.

Lagging univalents, most of which were dividing equationally (figs. 10, 11), were found at anaphase I in some sporocytes in each of the tetraploid clones, the frequency varying from 17.3 to 95.2% for the different clones. The daughter half-chromosomes from the lagging and dividing univalents began to separate at

late anaphase I (fig. 11) and were observed to be moving toward the poles at the end of anaphase I or early telophase I (figs. 12, 13). The infrequency of micronuclei in the cytoplasm during interphase compared with laggards at anaphase I indicated that a majority of the daughter half-chromosomes were included in the interphase nuclei. Rarely, a lagging univalent was observed in which the centromere apparently had not divided (fig. 13). These univalents were left in the cytoplasm at interphase (fig. 14).

Lagging univalents have been reported in several species to have resulted from the failure of metaphase I univalents to move to the poles at anaphase I. In this material frequency of anaphase I laggards was significantly correlated with metaphase I univalents ($r = 0.67$, $P < 0.01$). This value of r, although significant, is lower than that reported by MYERS (30) and MYERS and HILL (35) for plants of *D. glomerata*. Furthermore, in most tetraploid clones there was a higher frequency of sporocytes with laggards at anaphase I than with unpaired chromosomes at metaphase I (table 1). DARLINGTON (5, 6) and MYERS (32) have reported that laggards may result from incomplete disjunction of multivalents. If so, there might be expected a positive correlation between quadrivalent frequency and anaphase I laggards. In the tetraploid clones, r was 0.486 compared with r of 0.497 necessary for P of 0.05.

The daughter half-chromosomes from anaphase I laggards were observed to lag at anaphase II, and many were left in the cytoplasm to form chromatin clumps or—sometimes—micronuclei. The frequency of quartets with chromatin clumps and micronuclei was significantly correlated with frequency of laggards at anaphase I ($r = 0.548$, $0.05 > P > 0.02$).

EFFECTS OF INVERSIONS ON RANDOMNESS OF CHROMOSOMAL ASSOCIATION DURING PROPHASE

It has been proposed by MÜNTZING (22), DARLINGTON (7), and others that some species that behave cytologically as allopolyploids may have arisen from autopolyploids by a process of chromosomal differentiation. According to DARLINGTON (7), gene rearrangements rather than intragenic changes would be the principal factor in such differentiation. SKIRM (42) reported an autotetraploid *Tradescantia* with predominantly bivalent pairing that he attributed to structural heterozygosity and to doubling following fertilization. There arises, therefore, the problem of the relative importance of inversions in inhibiting the randomness of prophase pairing in autopolyploids. Dicentric chromatid bridges and acentric fragments that apparently had resulted from crossing-over in inversions were found at anaphase I in most plants of *D. glomerata* by MYERS and HILL (34). Such configurations were reported also by GILES (11) in autotetraploid *Tradescantia*. These observations indicate either that the inversion was present in the simplex or triplex condition, or that—if it was duplex—it did not limit synapsis to inverted and normal pairs in the chromosome section in which it occurred.

Bridges and fragments were observed at anaphase I in each of the diploid clones used in these investigations, the frequency varying from 0.74 to 11.12% of the sporocytes (table 2). Such configurations also were observed in each of the tetraploid clones. Since the inversions were duplex in the tetraploids, the results indicate that inverted segments

paired at prophase with homologous segments of normal chromosomes. There is the possibility that the colchicine treatment caused inversions in the tetraploid, which, being in the simplex condition, would be expected to pair with the homologous segment of one of the normal chromosomes. This seems unlikely, however, since the length of the dicentric chromatid and the size of fragment were similar in different sporocytes of the same clone and in the diploid and tetraploid clones of the same pair (figs. 15, 16), whereas considerable differences

TABLE 2

FREQUENCIES OF ANAPHASE I SPOROCYTES WITH DICENTRIC CHROMATID BRIDGE AND ACENTRIC FRAGMENT IN DIPLOID AND TETRAPLOID CLONES OF LOLIUM PERENNE AND THE FREQUENCIES EXPECTED IN THE TETRAPLOIDS, ASSUMING RANDOM PAIRING OF INVERTED AND NORMAL CHROMOSOMES WITH AND WITHOUT CORRECTION FOR DIFFERENCES IN CHIASMA FREQUENCY

Clone no.	X-ta of 4x in percentage of 2x	Percentage sporocytes with bridge plus fragment			
		2x observed	4x		
			Observed	Calculated	
				Uncorrected	Corrected for X-ta
	Field-grown material				
101	97.2	0.74	4.05	0.98	0.95
102	103.0	1.34	3.36	1.78	1.83
108	76.0	3.18	0.56	4.23	3.21
116	96.8	6.60	4.84	8.78	8.50
166	90.6	11.12	4.44	14.79	13.40
198	99.4	5.68	5.15	7.55	7.50
214	83.4	4.22	2.04	5.01	4.68
218	100.0	3.12	7.82	4.15	4.15
241	93.0	1.55	1.25	2.06	1.92
250	88.3	0.20	5.75	0.27	0.24
251	92.8	5.05	4.54	6.72	6.24
Av.		3.89	3.98	5.17	4.78
	Greenhouse-grown material				
50	91.8	2.07	10.86	2.75	2.52
101	104.2	3.39	6.26	4.51	4.70
183	105.9	2.26	3.18	3.00	3.18
198	96.4	4.66	12.86	6.20	5.98
218	103.3	4.05	11.04	5.39	5.57
Av.		3.29	8.82	4.37	4.39
Grand av.		3.70	5.49	4.92	4.66

were found among clones from different pairs (figs. 17, 18).

If normal and inverted segments pair at random during prophase in the autotetraploid, the frequency of sporocytes with bridges and fragments should be 1.33 times the frequency in the diploid, provided that chiasma formation occurs with equal ease. The observed frequencies of bridges and fragments, and those calculated with and without correction for differences in chiasma frequency at metaphase I in the tetraploid clones, are shown in table 2. In nine of the tetraploid clones the frequency of bridges and fragments was greater than calculated, while in seven it was less. The great variation in behavior observed in the different pairs may have resulted, in part at least, from the relatively small numbers of anaphase I sporocytes observed—an average of 148 per tetraploid clone and 223 per diploid clone. More data are required for a critical evaluation of the effects of inversions, but it is evident that inversions of the size found in this material did not appreciably inhibit randomness of chromosome pairing.

Discussion

The data indicate that there are three major types of meiotic irregularity in autotetraploid *Lolium perenne*, namely, (*a*) the members of the quadrivalents frequently disjoin unequally, resulting in 13–15 and 12–16—instead of the normal 14–14—distributions at anaphase I; (*b*) there are cases of incomplete disjunction of the members of quadrivalents resulting in lagging and dividing univalents at anaphase I; and (*c*) there is a high frequency of univalents at metaphase I that tend to lag and divide equationally at anaphase I and to be left in the cytoplasm at telophase I or telophase II. Each of these kinds of irregularity has been found in other autopolyploid species. Unequal disjunction from quadrivalents has been reported commonly (6, 7, 3, 17, 18, 20, 45, 46, 39, 43, and others), while relatively little attention has been given to the importance of unpaired chromosomes at metaphase I. This latter factor has been emphasized particularly by MYERS and HILL (35) and MYERS (30), who found it to be the most important kind of irregularity in *Dactylis glomerata*. The importance of improper disjunction from quadrivalents as a source of anaphase I laggards was indicated by COOPER'S (4) results with induced autopolyploid *Medicago sativa*. The results of the present investigations are in agreement with MYERS' (30) conclusions that the feature of meiotic irregularity of greatest importance varies with the species and that multivalent frequency alone will not always be a reliable criterion of stability and fertility of an autotetraploid.

Experimentally produced autopolyploids have, in general, been low in fertility. As stated previously, DARLINGTON (7), KOSTOFF (14), and many others have attributed this reduced fertility primarily to the formation during meiosis of multivalents that disjoin unequally at anaphase I. On the other hand, MÜNTZING (22) and RANDOLPH (40) have proposed that the infertility of autopolyploids results primarily from physiological disturbances and upsets in genic balance accompanying chromosomal reduplication. SPARROW, RUTTLE, and NEBEL (43), working with *Antirrhinum*, found differences in fertility among autotetraploids of different origin that were not associated with differences in quadrivalent frequency, and FISCHER (10) reported similar results from autotetraploids of maize. Likewise, MYERS and HILL (35) found that quadrivalent fre-

quency was not correlated with differences in fertility among plants of inbred progenies of *D. glomerata*. On the other hand, SPARROW *et al.* (43) reported that sterility was a concomitant of, but probably was not conditioned by, a significant difference in 18–14 distributions at anaphase I. MYERS and HILL (35) found that differences in fertility were significantly correlated with frequency of unpaired chromosomes at metaphase I, laggards at anaphase I, and micronuclei in the quartets in *Dactylis*. These features of meiotic irregularity accounted for approximately 16% of the variations in fertility, whereas MYERS (29) reported that about 38 and 23%, respectively, of the variability of seed set under bag and with open-pollination in that experiment could be attributed to heritable factors. Thus, it appears that meiotic irregularities and genetic differences both play a role in conditioning variations in fertility in *Dactylis*. It is significant, however, that variation in quadrivalent frequency did not have a measurable influence on fertility, yet multivalent frequency has been accepted commonly as a criterion of meiotic irregularity of autopolyploids. The inadequacy of such a measure is emphasized further by comparison of meiotic behavior of *D. glomerata* with that of autotetraploid *L. perenne*.

The infertility of experimental autopolyploids has led to the assumption (7, and others) that autopolyploids, as such, would seldom persist in nature as sexually reproducing species. DAWSON (8) concluded that autopolyploid species have persisted in nature by developing a mechanism giving regular disjunction at meiosis, or by developing some asexual type of reproduction.

In the perennial forage grasses, several species and chromosome races within species behave cytogenetically as autopolyploids. These include *D. glomerata* (21, 38, 33, 27), the tetraploid race of *Agropyron cristatum* (33), *Arrhenatherum elatius* (13, 33), *Anthoxanthum odoratum* (13), *Hordeum bulbosum* (2), and *Phleum pratense* (36, 24, 28, 31). These species are sufficiently fertile to permit their distribution commercially by seed. Of these, *D. glomerata* has been studied most extensively from the standpoint of meiotic behavior. Comparisons of meiosis in *Dactylis* and in autotetraploid *L. perenne* should provide evidence regarding some of the factors that have enabled *D. glomerata* to persist in nature as a sexually reproducing autotetraploid.

The range in quadrivalent and chiasma frequency among plants of *D. glomerata* is similar to the range among clones of autotetraploid *L. perenne*. On the other hand, unequal disjunction of the chromosomes of quadrivalents occurred commonly in *L. perenne* but was relatively infrequent in *D. glomerata*. Furthermore, improper disjunction of quadrivalents resulted in laggards at anaphase I in *L. perenne*, whereas MYERS and HILL (34, 35) found that quadrivalent frequency was not significantly correlated with frequency of anaphase I laggards in *D. glomerata*. And MYERS (30), using the analysis of covariance, showed that all significant differences in frequency of laggards could be attributed to variations in incidence of metaphase I univalents.

The differences in disjunction of quadrivalents in the two species may be attributed, in part at least, to differences in types of quadrivalents and in terminalization of the chiasmata. In *Dactylis* the quadrivalents were almost invariably simple rings and chains, while more complicated types were found in *Lolium*. The quadrivalents with symmetrical

arrangements might be expected to disjoin more regularly in a 2–2 manner at anaphase I. The complete terminalization of chiasmata in *Dactylis* probably also is a factor contributing to the greater regularity of disjunction of the quadrivalents, for—as DARLINGTON (7) has pointed out—quadrivalents with terminal chiasmata have a greater flexibility, permitting a more orderly orientation on the equatorial plate. Also, the simplest and quickest separation at anaphase I should be among the chromosomes with terminal chiasmata (7).

A further difference in meiotic regularity between *Dactylis* and *Lolium* autotetraploids is in the greater incidence of unpaired chromosomes at metaphase I in the latter. This difference cannot be attributed to lower chiasma frequency, since the two species were similar in that regard. It seems probable that the disturbance in timing relationships resulting from chromosome doubling in *Lolium* may have been an important factor in the higher univalent frequency.

One of the important features of the results obtained in these investigations was the lack of relationship between behavior of the diploid and of the related tetraploid clone. This lack was evident in the upset of timing in C108–4x compared with the other tetraploids, and in the insignificance of correlation coefficients between diploid and tetraploid clones in chiasma frequency, asynapsis at metaphase I, laggards at anaphase I, and micronuclei in the quartets.

Summary

1. Meiotic behavior was studied in pairs of diploid and tetraploid clones of *Lolium perenne* L. obtained by vegetative propagation from chimeral plants resulting from treatment of germinating seeds with solutions of colchicine.

2. As compared with the diploid clones, there was in the tetraploids a delay of chromosome contraction relative to the onset of metaphase I and in two clones absence of orderly orientation on the equatorial plate prior to initiation of anaphase I.

3. In some pairs the chiasma frequency of the tetraploid clone was lower than in the diploid, while in other pairs the differences were not significant.

4. There were three major types of meiotic irregularity in the tetraploid clones: (*a*) the members of the quadrivalents frequently disjoined unequally at anaphase I; (*b*) lagging and dividing univalents at anaphase I resulted in some cases from incomplete disjunction of quadrivalents; and (*c*) there was a high incidence of univalents at metaphase I that tended to lag and divide equationally at anaphase I and to be left in the cytoplasm at telophases I and II.

5. Variations in meiotic behavior among the tetraploid clones were not significantly correlated with variations among the diploid clones.

6. The frequencies of anaphase I sporocytes with dicentric bridges and acentric fragments in the tetraploid clones did not differ greatly from the frequencies calculated—assuming random pairing of inverted and normal chromosomes—from those obtained in the comparable diploid clones.

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STATE COLLEGE, PENNSYLVANIA

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EXPERIMENTAL POLYPLOIDY AND RUBBER CONTENT IN TARAXACUM KOK-SAGHYZ[1]

H. E. WARMKE

Introduction

Polyploidy is known to affect the storage of chemical products in many plants. Vitamin C content has been reported higher in polyploid than in diploid forms of the following species: apple (1), tomato (9), and rose hips (6). KOSTOFF and AXAMITNAJA (4) found more nitrogen and water in tetraploids than in related diploids of tomato. Pigment content of *Antirrhinum* and *Torenia* blossoms was found by STRAUB (10) to increase with the degree of polyploidy (2n, 3n, 4n, 6n, and 8n). STRAUB also demonstrated that the carotene content per cell in the yellow spot of blossoms of the latter genus increases from diploid through octoploid, the increase in pigment content being greater than the relative increase in cell volume. RANDOLPH and HAND (8) reported that doubling of chromosome number in yellow corn causes a 40% increase in carotenoid content. Triploid and tetraploid forms of hemp have a higher marihuana toxicity per unit weight of dry leaves than related diploids (12, 14). Recently, GUSTAFSON (3) has reported an opposite effect in marigold and the cherry tomato; namely, that tetraploids have less growth hormone than the diploids from which they were derived.

Polyploidy has also been shown by numerous workers to effect an increase in the dimensions of plant structures. In general, tetraploids differ from diploids in having thicker stems, broader leaves, larger flowers, thicker roots, larger pollen grains and seeds, etc. It seemed of value, therefore, to induce chromosome doubling in *Taraxacum kok-saghyz*, the Russian dandelion, and to study the effect of such doubling on rubber yield. The rubber in this species is in the latex of the root, which—as in our domestic species—is a more or less branched tap root. An increase in the percentage of rubber or an increase in the size of the root, or

[1] Co-operative project with Rubber Plant Investigations, U.S. Department of Agriculture, Agricultural Research Administration, Bureau of Plant Industry, Soils, and Agricultural Engineering.

both, would be desirable characteristics from the standpoint of rubber yield.

Polyploidy in the genus *Taraxacum* is also of theoretical interest in relation to apomixis. In this genus, diploid species reproduce in the normal sexual manner; natural polyploid species, on the other hand, are apomictic. On the female side of the polyploids there is a failure of one of the meiotic divisions (diplospory, **2**), resulting in the formation of an unreduced egg. This egg undergoes development without fertilization (diploid parthenogenesis). It was thus of interest to observe the effect of autopolyploidy on reproduction in *T. kok-saghyz*, which is a sexual diploid with chromosome numbers of n = 8, 2n = 16.

The Russian workers KOSTOFF and TIBER (**5**) and NAVASHIN and GERASSIMOVA (**7**) obtained tetraploid plants of this species by colchicine treatment. They did not report changes in rubber percentage or in root size, and they apparently observed no effect on the reproductive processes in the tetraploid forms. Many workers in this country, including Drs. G. L. STEBBINS, L. F. RANDOLPH, and ERNST ARTSCHWAGER, also have succeeded in obtaining tetraploids in this species by colchicine treatment (personal communications).

MATERIAL AND METHODS.—Seeds of *T. kok-saghyz* were received from the U.S. Department of Agriculture, Rubber Plant Investigations, on May 16, 1942, under plant quarantine no. 143960. These were treated by immersion for 1, 2, and 4 days in 0.05%, 0.1%, 0.2%, 0.4%, and 0.8% aqueous solutions of colchicine in covered Syracuse dishes at room temperature. The seeds were rinsed in water before being planted in seed pans in the greenhouse. Surviving seedlings were transplanted to 3-inch and later to 4-inch pots.

Successfully treated plants were identified by microscopic examination of pollen grains. Pollen determinations were subsequently checked by making chromosome counts from root tips of many second-generation polyploid plants, using the section-smear technique (**11**).

Roots were prepared for rubber analysis in the following manner: they were dug, carefully washed, immersed in boiling water for 20 seconds, and then decapitated at the level of the crown. Immersion in boiling water was used at the suggestion of Dr. RANDOLPH, to coagulate the latex and thus prevent "bleeding" when the top was removed. The roots were then blotted with paper towels to remove excess water and weighed. They were allowed to dry at room temperature until a constant weight was reached and then were reweighed to determine the relative loss of weight in drying. Rubber analyses were made by Dr. SAM WILDMAN of the Department of Agriculture, using a turbidimetric method.

Results

The results of treatment of seeds of *T. kok-saghyz* are given in table 1. Of the 123 treated plants which flowered, nineteen were found to be tetraploid or predominantly tetraploid, on the basis of pollen size. Pollen of untreated controls and of unsuccessfully treated plants averages about 28 μ in diameter, that of induced tetraploids about 36 μ (fig. 1). In addition to having larger pollen grains, successfully treated plants usually also are distinguishable by their thicker, broader leaves, thicker flower stalks, and larger buds and flowers.

Table 1 shows a decrease in the number of plants surviving, both with increasing concentration of colchicine and with duration of treatment. Few plants survived concentrations of 0.4% and

0.8%; also, relatively few survived the 4-day treatment. The highest percentages of successfully treated plants (27.3% and 44.4%, respectively) were obtained from treatments in 0.1% and 0.2% colchicine solutions for 2 days.

T. kok-saghyz is highly self-sterile and rarely sets seed in a screened greenhouse (13); this is true for diploids and tetraploids alike. Abundant seed is secured, however, when flower heads from two different plants are rubbed together. Chromosome counts were made from root tips of many of the plants to verify the pollen determinations. Diploid, triploid, and tetraploid figures are shown in figure 4.

The second-generation tetraploid seedlings show the usual polyploid characters, but to an extreme degree (fig. 3). Their leaves are broader and shorter than the corresponding diploids; they also are thicker and somewhat less deeply incised. The tetraploids produced

TABLE 1

RESULTS OF TREATMENT OF SEEDS OF T. KOK-SAGHYZ IN AQUEOUS SOLUTIONS OF COLCHICINE; 75–100 SEEDS TREATED IN EACH INSTANCE. PLANTS WHICH FLOWERED WERE CHECKED FOR CHROMOSOME DOUBLING BY POLLEN DETERMINATIONS

COLCHICINE CONCENTRATIONS (%)	DURATION OF TREATMENT											
	One day				Two days				Four days			
	No. plants surviving	Plants flowering			No. plants surviving	Plants flowering			No. plants surviving	Plants flowering		
		2n	4n	% 4n		2n	4n	% 4n		2n	4n	% 4n
0.05	58	9	1	10.0	66	30	3	9.1	33	8	.....	.....
0.1	70	20	2	9.1	54	16	6	27.3	11	1	.....	.....
0.2	42	12	3	20.0	30	5	4	44.4	5	.....	.....	.....
0.4	4	.....	.....	.....	5	2	.....	.....	.....	.....	.....	.....
0.8	4	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total	178	42	6	12.5	155	53	13	19.7	49	9	.....	.....

The seed from tetraploid crosses is clearly larger than that from diploid crosses (fig. 2).

Many crosses were made among the original induced tetraploids and between these and untreated diploids. From such crosses, tetraploid, triploid, and diploid seed was obtained. Part of the tetraploid seed was forwarded to R. W. HENDERSON at the U.S.D.A. Rubber Plant Field Laboratory, St. Paul, Minnesota; the remainder, together with triploid and diploid seeds, was sown in seed pans in the greenhouse at Cold Spring Harbor. larger and more leafy rosettes than the diploids throughout the period of these tests.

For comparison of root weight and rubber content, two different lots of seed were planted: the first was small, consisting of seed from six diploid and three tetraploid crosses; it was started 12/19/42 and was grown until time of harvest (7/10/43) in pots in the greenhouse. Mean rubber percentage and fresh root weights for nineteen diploid and nineteen tetraploid plants from this planting are given in table 2.

The second comparison between diploids and tetraploids was started 4/30/43 and concluded 12/1/43. This included a greater number of plants (thirty-six diploids and forty-three tetraploids), which, after being started in the greenhouse, were transplanted to a small experimental plot outdoors. Each plant was given approximately 1 square foot of space. In addition to root weight and rubber percentage, this group was compared also as to water content of the roots, by weighing roots both fresh and dry. Comparisons of the field-grown diploids and tetraploids are given in table 2.

Mean fresh-root weight is greater for tetraploids than for diploids in both the greenhouse and the field comparisons. Even though the numbers are small, the differences are significant in both cases (diff./s.e. = 2.14 and 3.65, respectively). Among the field plants, diploid roots lost 73.23 ± 0.49% by weight in drying, and the tetraploids lost 72.45 ± 0.57% (table 2). These differences are not significant (diff./s.e. = 1.04) and indicate a closely comparable water content for 2n and 4n roots of this species.

The results of rubber analyses differ under the two sets of conditions. The greenhouse comparisons indicate a lower percentage of rubber in the tetraploids than in the diploids. This difference approaches significance (diff./s.e. = 1.91). The field-grown tetraploids exceed the field-grown diploids both in root weight and in rubber percentage. In this case the difference between diploid and tetraploid in rubber percentage is clearly significant (diff./s.e. = 3.90). When data for both greenhouse and field-grown plants are combined (table 2), the tetraploids are seen to have significantly higher mean root weights (16.77 ± 1.68 gm. to 9.21 ± 0.93 gm.) and a somewhat higher rubber percentage (2.95 ± 0.28% to 2.14 ± 0.26%). These differences are probably both significant (diff./s.e. = 3.94 for root weight and 2.13 for rubber percentage). The largest single fresh root observed, 56.5 gm., was a tetraploid; the highest individual rubber percentage, 8.9%, occurred in a diploid.

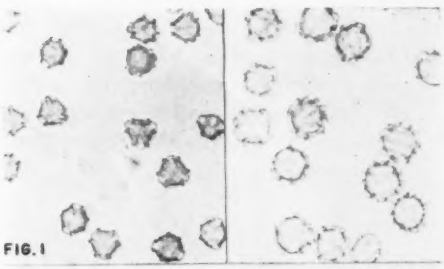
FIG. 1

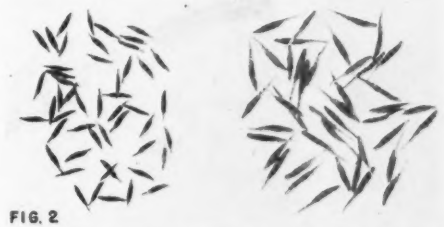
FIG. 2

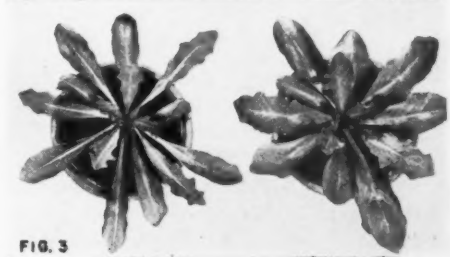
FIG. 3

FIGS. 1–3.—Comparison of diploid and tetraploid plants of *T. kok-saghyz*. Diploid at left and tetraploid at right: Fig. 1, pollen grains. Fig. 2, seeds. Fig. 3, seedlings, age 68 days.

What is of greater importance ultimately than either root weight or rubber percentage, taken separately, is their combination; that is, how much rubber a tetraploid plant yields as compared with a diploid. Rubber yields have been cal-

culated for these plants by multiplying dry-root weights in grams by rubber percentages of individual roots (table 2). The combined diploids produced an average of 0.060 ± 0.009 gm. of rubber per plant; the combined tetraploids produced an average of 0.195 ± 0.035 gm., nearly 3.3 times as much.

To be a high yielder it is obvious that a plant must have both a large root and one containing a high percentage of rubber. The correlations between root weight and rubber percentage are therefore important. These are given in table 3. There is some positive correlation between these characters in all cases except the greenhouse tetraploids, where a low negative correlation is obtained. Since the numbers are small, however, these values are of questionable significance.

In table 4, dry-root weights, rubber percentages, and yields for the ten best diploids and the ten best tetraploids are recorded. These comparisons show that the most productive tetraploid produced more than four times as much rubber as the most productive diploid (1.131 to 0.263 gm.). The poorest of the ten tetraploids produced more rubber than the best diploid. The higher correlation coefficient (0.561) between root weight and rubber percentage in the field-grown tetraploids is clearly evident in table 4.

In contrast to the natural polyploids of this genus, the colchicine-induced polyploids show no signs of apomictic reproduction. Reproduction is proved to be sexual by results of the 4n × 2n and reciprocal (2n × 4n) crosses. Triploids, with chromosome numbers of 24, have been obtained from both crosses. In apomictic species the chromosome number of the offspring is that of the female parent, regardless of the pollen used. Pollen of the experimental tetraploids is well formed (fig. 1) and viable, as indicated by its functioning on diploid females to produce triploid offspring. Pollen of natural polyploids, although most likely a result rather than a cause of apomixis, is invariably aborted to a high degree.

Colchicine-induced polyploids of *T. kok-saghyz* are highly self-sterile, as are diploids, and set seed very rarely when grown in a screened greenhouse. No accurate data are available on the degree of relative self-sterility of diploids and tetraploids of this species, but the in-

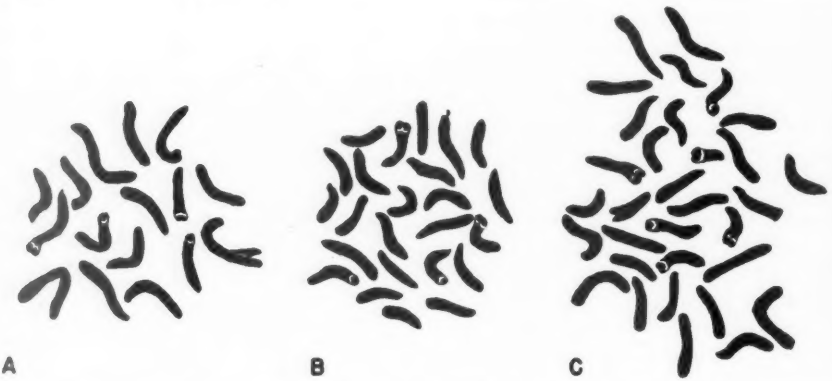

FIG. 4.—Chromosomes from root tips of polyploid plants of *T. kok-saghyz: A*, diploid (2n = 16); *B*, triploid (3n = 24); *C*, tetraploid (4n = 32).

TABLE 2

RELATIVE WEIGHTS, RUBBER PERCENTAGES, AND CALCULATED RUBBER YIELDS OF DIPLOID AND TETRAPLOID ROOTS OF T. KOK-SAGHYZ

Material	No. of plants	No. diff. families	Mean fresh-root wt. (gm.)	Range mean fresh-root wt. (gm.)	Mean dry-root wt. (gm.)	Loss of wt. in drying (%)	Mean rubber, dry roots (%)	Range rubber, dry roots (%)	Calculated rubber yield† per plant (gm.)
Greenhouse plants 2n	19	6	8.23 ± 0.74	4.13–17.62	2.20 ± 0.20*	.....	2.13 ± 0.51	0.0 –8.9	0.053 ± 0.013
Greenhouse plants 4n	19	3	10.71 ± 0.89	4.41–21.20	2.95 ± 0.25*	.....	1.04 ± 0.26	0.0 –4.4	.029 ± .007
Diff./s.e.	.....	.....	2.14	.....	2.34	.....	1.91	.....	1.60
Field plants 2n	36	12	9.73 ± 1.37	0.5 –31.4	2.72 ± 0.40	73.23 ± 0.49	2.15 ± 0.29	0.15–6.9	.063 ± .012
Field plants 4n	43	13	19.45 ± 2.28	2.35–56.5	5.71 ± 0.73	72.45 ± 0.57	3.79 ± 0.30	0.15–7.1	.208 ± .047
Diff./s.e.	.....	.....	3.65	.....	3.60	1.04	3.90	.....	4.18
Combined greenhouse and field plants 2n	55	18	9.21 ± 0.93	0.5 –31.4	2.54 ± 0.27	.....	2.14 ± 0.26	0.0 –8.9	.060 ± .009
Combined greenhouse and field plants 4n	62	16	16.77 ± 1.68	2.35–56.5	4.87 ± 0.54	.....	2.93 ± 0.28	0.0– 7.1	0.195 ± 0.035
Diff./s.e.	.....	.....	3.94	.....	3.88	.....	2.13	.....	3.75

* Calculated from fresh-root weights, assuming same relative water loss as in field plants.

† Rubber percentage × dry-root weight (gm.), individual plants.

frequency of seed-setting in both forms in a screened greenhouse indicates that neither is apomictic. Plants of two naturally occurring polyploid and apomictic species, *T. officinale* and *T. megalorrhizon*, when grown in the same greenhouse with *T. kok-saghyz*, produced a full set of seed in every flower head.

TABLE 3

CORRELATION COEFFICIENT OF FRESH ROOT WEIGHT VS. RUBBER PERCENTAGE FOR 2n AND 4n PLANTS OF T. KOK-SAGHYZ

Plants	Greenhouse	Field
2n	0.327±0.205	0.118±0.164
4n	−0.117±0.226	0.561±0.105

The experimental triploids, probably because of irregularities in chromosomal disjunction, are highly cross-sterile as well as self-sterile. These forms essentially are capable of vegetative propagation only. If apomixis were a direct response to decreased sexual fertility of polyploids, it would be expected to appear in these triploid individuals. That it does not indicates that the cause of apomixis is more complex and apparently involves a series of evolutionary steps. The fact, however, that—apparently without exception—the many natural polyploids are apomictic points to polyploidy as an essential to the evolution of apomixis in this genus.

Discussion

From the standpoint of rubber yield, these comparisons place the tetraploid form in a very favorable position. Its roots are significantly larger, and its rubber percentage is equal to or slightly superior to that of diploids. The lower percentage of rubber in the greenhouse-grown tetraploids probably should not be taken too seriously, for the following reasons: (*a*) The number of plants in the greenhouse tests is small; (*b*) harvesting was done in July, when rubber storage is known to be low and erratic; and (*c*) the plants were pot-grown, and therefore grew under unfavorable conditions. The negative correlation between root size and rubber percentage for this particular group of plants may indicate that greenhouse conditions adversely affect

TABLE 4

CALCULATED RUBBER YIELDS FOR TEN MOST PRODUCTIVE DIPLOID AND TEN MOST PRODUCTIVE TETRAPLOID PLANTS OF T. KOK-SAGHYZ

Rank	Diploid			Tetraploid		
	Dry root wt. (gm.)	Rubber (%)	Calculated rubber yield (gm.)	Dry root wt. (gm.)	Rubber (%)	Calculated rubber yield (gm.)
1	8.35	3.15	0.263	16.76	6.75	1.131
2	6.49	3.75	0.243	15.43	6.30	0.972
3	2.51	8.90	0.223	14.30	5.10	0.729
4	4.63	3.60	0.167	15.95	4.05	0.646
5	2.48	6.30	0.156	9.87	5.55	0.548
6	2.30	6.50	0.150	9.41	5.70	0.536
7	7.82	1.80	0.141	9.52	5.40	0.514
8	1.63	6.90	0.112	9.89	4.05	0.401
9	3.11	3.50	0.109	6.50	5.40	0.351
10	4.20	2.25	0.095	7.57	4.34	0.329
Mean	4.35	4.67	0.166	11.52	5.26	0.616

rubber storage in the larger-rooted tetraploids.

The total number of plants studied in these tests is not large—certainly not so large as one would like for a comparison of diploids and tetraploids in a species as heterozygous as *T. kok-saghyz*. However, an effort was made to draw the plants from as wide a base as possible: eighteen families are represented among the fifty-five diploids and sixteen families among the sixty-two tetraploids. The usual method of obtaining comparable 2n and 4n samples from heterozygous stock—that of treating part of the plant with colchicine and then collecting seeds from both the diploid and tetraploid portions—is not applicable in a self-sterile species such as this. If diploid and tetraploid blossoms were obtained on the same plant, these would have to be outcrossed, and the favorable basis of comparison would thus be lost. If rare cases of selfing could be achieved, the problem still would not be solved, for such offspring would almost surely suffer deleterious effects from inbreeding, perhaps differentially.

The problem of relative set of seed in diploid and tetraploid stocks under field conditions has not been studied adequately. This is an important practical consideration, of course, since the fertility of tetraploids is usually lower than that of diploids. Dr. STEBBINS reports a relatively high set of seed but poor fertility from his 4n stocks of *T. kok-saghyz* (personal communication). Our experience with hand-pollinations of greenhouse plants, however, indicates a high degree of fertility among the tetraploid plants, possibly almost as high as among the diploids. The ease with which *T. kok-saghyz* can be propagated vegetatively, however, may make high fertility of less importance in this species than in some others.

The tetraploid plants appeared to suffer less from the phenomenon of "summer dormancy" in these trials than did the diploids. The 4n rosettes were larger and leafier and showed better growth during the summer months than the 2n rosettes. Whether the tetraploids will maintain this advantage in vegetative vigor under more favorable conditions is not known; admittedly the climate and soil of Long Island are not the best for culture of this species.

It is evident that the preceding results should be interpreted as suggesting, rather than as proving, superiority of the tetraploid as a rubber producer in this species. Large-scale field trials, under optimum conditions, would seem to be desirable.

Summary

1. Diploid and tetraploid races of *Taraxacum kok-saghyz* have been obtained by colchicine treatment.

2. Nineteen greenhouse-grown tetraploids had significantly larger roots but a lower rubber percentage than a similar number of greenhouse-grown diploids. Forty-three field-grown tetraploid plants had both significantly larger roots and higher rubber percentage than a group of thirty-six comparable diploids. Combined data from greenhouse and field tests show the tetraploids to have larger roots (16.77 ± 1.68 gm. to 9.21 ± 0.93 gm., fresh weight) and a somewhat higher rubber percentage ($2.95 \pm 0.28\%$ to $2.14 \pm 0.26\%$) than the diploids.

3. The water content of diploid and tetraploid roots did not differ significantly.

4. A low positive correlation between root weight and rubber percentage was observed for field-grown diploids and tetraploids, and for greenhouse-grown diploids. A slight negative correlation was observed for the greenhouse-grown tetraploids.

5. On the basis of calculated rubber yield (dry weight of roots × percentage of rubber), the diploids produced an average of 0.060 ± 0.009 gm. of rubber; the tetraploids an average of 0.195 ± 0.035 gm., or more than three times as much. The best single tetraploid produced more than four times as much rubber as the best single diploid.

6. No indication of apomixis was observed among the experimental triploids or tetraploids in these studies.

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NATURE AND RATE OF DEVELOPMENT OF ROOT SYSTEM OF GONOLOBUS LAEVIS[1]

JOHN C. FRAZIER

Introduction

This is the fifth of a series of reports on growth habits of noxious perennial weeds of central United States being conducted by the Kansas Agricultural Experiment Station. A study (2) of the nature and rate of development of field bindweed established the scope and methods of procedure. The second investigation (3) dealt with hoary cress, the third (4) with Russian knapweed, and the fourth (5) with dogbane. The present study is concerned with climbing milkweed, *Gonolobus laevis* Michx., a rather important weed pest locally abundant in certain cultivated fields of the eastern two-thirds of the state of Kansas. Its nature as a weed in Kansas has not changed in recent years, HITCHCOCK and CLOTHIER (8) having described it in 1898 as "not widespread in Kansas but locally very abundant. Quite a pest in corn fields in

[1] Contribution no. 464, Department of Botany, Kansas Agricultural Experiment Station.

certain localities." According to GATES (6) the weed has not spread westward appreciably in the state in the last 40 years. GATES (personal communication, 1944) believes it is becoming more abundant within its area of distribution, particularly in central Kansas.

Environmental conditions and methods

SOIL DATA.—All plants[2] considered in this paper were taken from soil, the profile of which is essentially the same as that described by FRAZIER (2), except that it has two narrow layers of compacted material which may have influenced water penetration. These layers, each approximately 1 inch thick, were at the 26- and 32-inch depths. Both profiles were described by Dr. J. C. HIDE of the Kansas Agricultural Experiment Station.

METEOROLOGICAL DATA.—The monthly and annual temperature and precipitation data for the first 10 months of 1944 compared with the long-time average of these factors are given in table 1. The summer of 1944 had maximum temperatures of 100° F. or higher on 4 days, as compared with 38 such days in 1937, when the comparable study was made on field bindweed; 15 in 1941, when the comparable study was made on hoary cress; 5 in 1942, when the comparable study was made on Russian knapweed; and 9 in 1943, when the comparable study was made on dogbane. The average annual number of days having temperatures of 100° F. or higher during the 50 years, 1889–1938, is 15. The average monthly precipitation for the first 10 months of 1944 was 4.40 inches, as compared with the monthly average of 2.86 inches for these months during the 83-year period, 1858–1940. The total annual rainfall for 1944 was 49.57 inches, only 1.25 inches below the maximum record of 50.82 inches in 1915. Some of the precipitation for the first 10 months of 1944 fell as heavy rains. There were two 24-hour periods in April with 1.94 and 1.96 inches, one in May with 1.79 inches, one in July with 1.82 inches, two in August with 1.84 and 5.86 inches, and one in September with 2.03 inches—making a total of seven such periods, each with a rainfall of more than 1.75 inches.

TABLE 1

MONTHLY AND ANNUAL TEMPERATURE (IN °F.) AND PRECIPITATION (IN INCHES) AT MANHATTAN, KANSAS, 1944

MONTH	TEMPERATURE		PRECIPITATION	
	Mean*	85-year average 1856–1940†	Total*	83-year average 1858–1940†
Jan.	35.9	27.0	1.00	0.75
Feb.	35.2	31.5	.85	1.14
Mar.	38.1	42.5	4.52	1.48
Apr.	49.9	54.5	8.92	2.67
May	68.4	64.6	3.69	4.31
June	75.5	74.5	2.92	4.50
July	77.3	79.5	6.81	4.25
Aug.	79.0	77.5	11.65	3.89
Sept.	68.7	69.3	2.53	3.44
Oct.	58.5	56.5	1.15	2.16
Annual mean or total	55.2	54.2	49.57	30.97

* Meteorological data obtained from U.S. Weather Bureau, Kansas Section.

† Computed by Dr. A. B. CARDWELL of Kansas State College.

METHODS.—The plants were grown on a small plot of land free from other noxious weeds and from sodium chlorate. The soil is a fairly typical Geary silt loam representative of the wind-deposited soil of the upland of this locality. The plot had been cultivated a decade earlier but had not been disturbed recently.

Seeds of climbing milkweed were

[2] The term plant as used in this paper refers to all the growth from a single seed. The term shoot is applied to the individual leafy stems commonly called plants in control studies.

planted on May 5, 1944. Fifteen seeds were placed $\frac{1}{4}$ inch below the surface at each of seventeen points which were so spaced that six of the plants were 14 feet distant from any other and the remainder were separated by 8 feet. May 19, 1944, was taken as the date of emergence for the series, since one seedling emerged at each of fourteen planting points on that date; all seedlings emerging before and after were removed. The area was kept free of all other vegetation, so the only competition for water and plant nutrients, if any existed, was among the plants of the milkweed.

The root systems were excavated at intervals ending November 10, 1944, by a modification of the trenching method developed by WEAVER (10) as described by FRAZIER (2). Efforts were made to keep the root system as one organic entity; if a root broke, the two ends were immediately tied together. Measurements of the plant parts were made and recorded, so that as illustrated they occupy the same relationship, one part to another, as they did in the soil, except that in figures 1, 2, 3, 4, and 6 all lateral roots are arranged in one plane. While as many as possible of the finer roots were obtained, it is not contended that a large portion of them was secured.

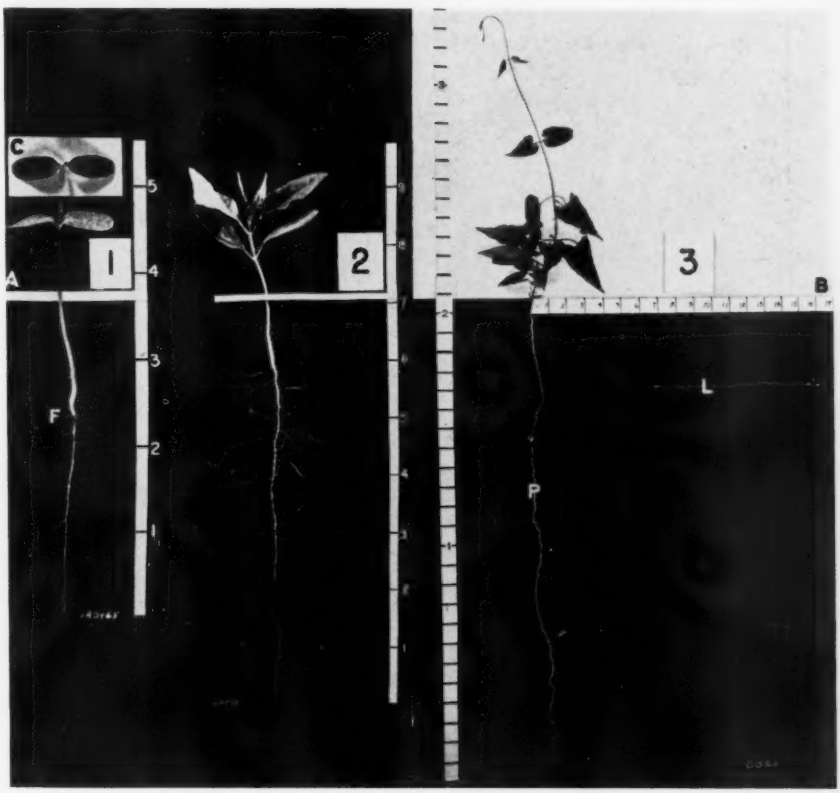
Observations

The rate of plant development under known soil and climatic conditions was observed by means of seven plants excavated 1, 2, 3, 5, 9, 13, and 25 weeks after emergence. The dates of excavation were May 26, June 2, June 9, June 23, July 21, August 18, and November 10, 1944. The plants are designated 1–7, inclusive. Figures 1, 2, 3, 4, and 6 show the vertical penetration and radial spread of roots of representative plants. Data on the plants are given in table 2.

By 5 weeks after emergence the general plan of the root system was becoming established (fig. 3). Plant no. 6, taken the thirteenth week after emergence (fig. 4), showed particularly well the gross morphological nature of the root system. A taproot rapidly penetrated directly downward from the germinating seed. It was a primary root in order of development and vertical in position; hence, it is designated the primary vertical root (fig. 4*P*). Many branch roots arose throughout the length of this taproot, most of them small feeding roots. A few, probably those more favorably situated in relation to soil moisture and plant nutrients, grew extensively and became permanent laterals (fig. 4*L*). They are designated permanent lateral roots of the first order. These roots tended to grow as radii about the primary vertical root in a plane parallel to the soil surface in the upper 15 inches (more commonly the upper 6 inches) of the soil. They gave rise to branch roots over any part of their growth. Most of them were small feeding roots. Some, however, developed into branch lateral roots, which are designated as permanent lateral roots of the second order (fig. 4*H*). They grew horizontally, at right angles to the root on which they arose, for a distance of approximately 1–5 inches, then the majority grew horizontally away from the primary vertical root. In a similar manner permanent lateral roots of the third order arose on those of the second, and those of the fourth order on those of the third, etc. This branching tended to develop in the area between the permanent laterals of the first order. The result was that the plant occupied a much higher proportion of an ever-increasing area, somewhat circular in shape, than it would have done otherwise. The permanent laterals of the first order outgrew those of the other orders, however, so that they ex-

tended a greater horizontal distance from the primary vertical root. Concurrent studies indicated that plants subject to severe competition made less rapid horizontal development.

arose on the sides, and rarely on the top, of the permanent lateral roots of any order, in which case they grew horizontally 1–7 inches and then bent downward to become vertical taproots (fig. 6*T*). Both



FIGS. 1–3.—Development of plants of climbing milkweed. *A–B*, ground line common to all three figures. Fig. 1, seedling 1 week after emergence; *C*, view of cotyledons from above; *F*, root-stem transition zone. Scale in inches. Fig. 2, young plant 3 weeks after emergence showing two pairs of true leaves above cotyledons. Scale in inches. Fig. 3, young plant 5 weeks after emergence showing characteristic development of nutating stem which has commenced to twine. Note typical size and shape of leaves and relative length of internodes. *P*, primary vertical root; *L*, permanent lateral root. Vertical scale in feet and inches; horizontal scale in inches.

In addition to the primary vertical root (figs. 4*P*, 6*P*), other vertical roots were formed. These usually arose on the lower side of a permanent lateral of any order, from which they grew directly downward (fig. 6*S*). Less commonly they types are designated secondary vertical roots. While these vertical roots arose on permanent laterals of any order, they naturally arose first on permanent lateral roots of the earlier-formed orders.

All shoot development, except that

from the plumule, was derived from root-borne buds. In the one growing season of 25 weeks, three root-borne buds were produced on permanent lateral roots of the third order, nine on those of the second order, and seventeen on those of the first order. None was produced on the secondary vertical roots of plants not over 25 weeks old, but they were found on all primary verticals observed of that age. The number of these buds in the surface 15 inches of such roots ranged from four to thirty-one. The latter appeared to be an exceptional case, and the primary vertical root in this instance had but two poorly developed permanent laterals of the first order, one of which did not exceed $2\frac{1}{2}$ feet in length while the longer attained approximately 5 feet.

By 10 weeks after seedling emergence, several of these buds had been formed on the permanent lateral roots of the first order 12–18 inches from the primary vertical root. Thirteen weeks after seedling emergence such buds had given rise to rhizomes, but these had not emerged (fig. 4*R*). Figure 5 shows in some detail the rhizome shown at *R* in figure 4. Nodes are not readily distinguishable on the rhizomes of climbing milkweed as they are on those of field bindweed, hoary cress, Russian knapweed, and dogbane, because the small evanescent scale leaves are soon lost and the buds at the nodes, if present, are not prominent. Emergence of rhizomes first occurred 15 weeks after seedling emergence. The above-ground portion of a rhizome is a leafy shoot. The root-borne stem buds gave rise to rhizomes unless they were borne at the ground line, in which case they gave rise to leafy shoots.

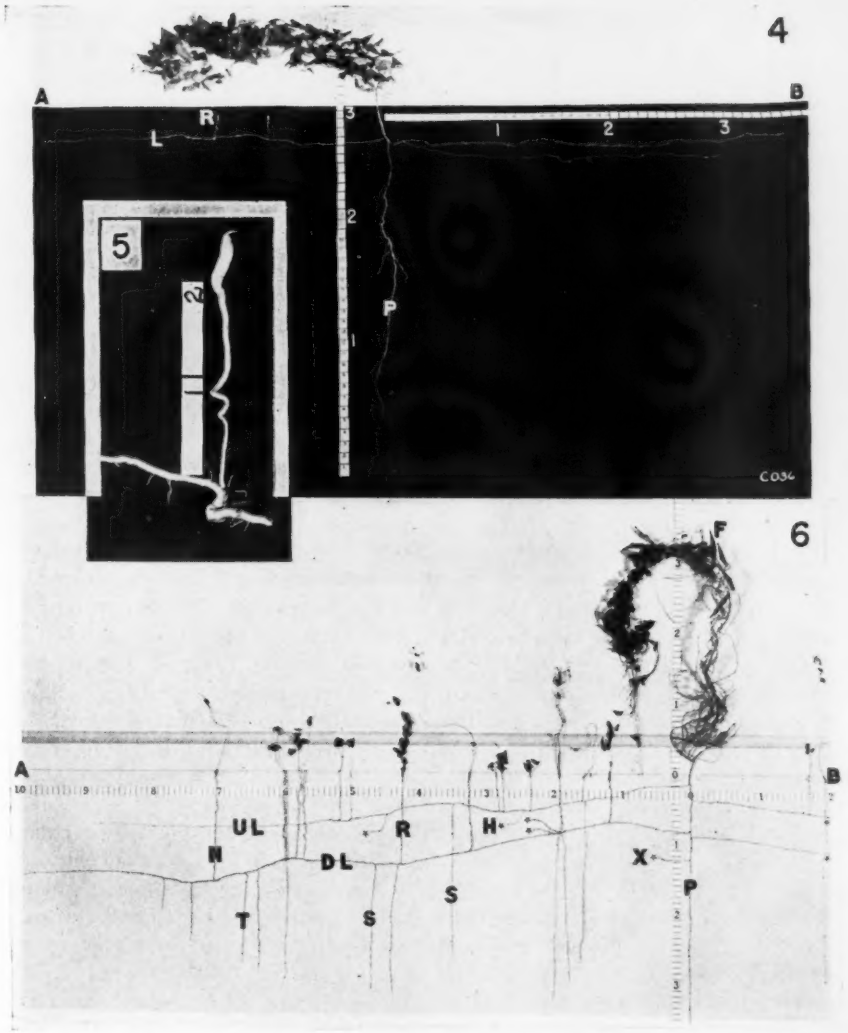
Twenty-five weeks after seedling emergence, a plant (fig. 6) had sent its primary vertical root (*P*) to a depth of 42 inches and had produced four permanent lateral roots of the first order which were arranged radially around the primary vertical. Two of these permanent laterals of the first order had formed

TABLE 2

RATE AND NATURE OF DEVELOPMENT,* AFTER SEEDLING EMERGENCE, OF ROOTS OF CLIMBING MILKWEED PLANTS, MANHATTAN, KANSAS, 1944

Plant no.	Weeks after emergence	Maximum vertical penetration (inches)	Maximum radial spread (inches)	Plant condition
1	1	$3\frac{1}{2}$	$\frac{5}{8}$	Typically shaped cotyledons (fig. 1)
2	2	$5\frac{1}{8}$	$1\frac{1}{4}$	First true leaves
3	3	7	$2\frac{3}{8}$	Second pair of true leaves (fig. 2)
4	5	$10\frac{1}{4}$	$6\frac{1}{8}$	Stem axis beginning to elongate
5	9	$24\frac{3}{4}$	16	Later-formed internodes greatly elongated, forming characteristic nutating stem; latter, which was 16 inches long, had begun to twine. Permanent lateral roots well established (fig. 3)
6	13	$36\frac{1}{2}$	$44\frac{1}{2}$	Main stem 42 inches long and well supplied with leaves. Root system well established. Two well-formed rhizomes on one permanent lateral root (fig. 4)
7	25	42	119	Main stem 96 inches long bearing 16 fruits. Permanent lateral root of first order extending greatest distance from primary vertical root had produced 9 rhizomes, each of which bore a shoot, 8 secondary vertical roots, and 2 lateral roots of second order which branched further (fig. 6)

* First flowering occurred July 29, 1944.



FIGS. 4–6.—Plant parts arranged to show essentially same relationship as in the soil. *A–B*, ground line. Fig. 4, complete plant 13 weeks after seedling emergence: *P*, primary vertical root; *L*, permanent lateral root of first order; *R*, rhizome. It had grown approximately two-thirds the distance to the soil surface. Scale in feet and inches. Fig. 5, same rhizome reversed and enlarged. Bend in lateral root has no relation to rhizome formation. Note gradual increase in size from point of origin on lateral root ending in enlargement near tip. Scale in inches. Fig. 6, portion of a plant 25 weeks after seedling emergence. Primary vertical root (*P*) subtending extensively developed shoot; portions of two permanent lateral roots to right of that root; and approximately 80% of root and shoot development of the two longest, typical permanent lateral roots of the first order. The one (*UL*) developed in soil that had not been disturbed in recent years; the other (*DL*) grew 4 feet into soil which had been removed to a depth of 2 feet 2 years earlier, then replaced (each foot level in proper place) at that time. *S*, secondary vertical root of type more commonly found, originating on lower side of permanent lateral root; *T*, secondary vertical root of less commonly found type originating on side of permanent lateral root; *H*, permanent lateral root of second order (there are two on *UL* and two on *DL*); *X*, stub of a permanent lateral root of first order; *N*, older rhizome showing development at that stage; *F*, fruit. Stars indicate points where roots have been removed for clarity. Many leaves, particularly on shoots of the older rhizomes, were lost before picture was taken. Scale in feet and inches.

permanent laterals of the second and higher orders. One had a permanent lateral of the fourth order. Three of the permanent lateral roots of the first order grew in an undisturbed soil comparable with that of the other studies of this series (**2**, **3**, **4**, **5**). The lateral root (fig. 6*UL*) is one of these three permanent laterals that grew in undisturbed soil. It had six rhizomes, each of which bore a shoot, two secondary vertical roots, and two permanent lateral roots of the second order, one of which had a permanent lateral root of the third order. The permanent lateral root (fig. 6*DL*) extended 4 feet of its total length of 10 feet into soil which had been excavated to a depth of 2 feet during root studies made 2 years earlier, then replaced—each foot level in place—at that time. This permanent lateral root was somewhat more extensively developed. It had nine rhizomes, each of which bore a shoot, eight secondary vertical roots, and two permanent lateral roots of the second order, of which both had permanent laterals of the third order and one had a permanent lateral of the fourth order.

Discussion

The rate of growth of plants of climbing milkweed from seed under known soil and climatic conditions, with little or no competition, is not to be construed as representing their growth rate under all conditions. It illustrates only the growth potential in a favorable noncompetitive situation.

HITCHCOCK and CLOTHIER (**7**), in a study on this weed, which they called *Enslenia albida* Nutt., observed a vertical root which extended 7 feet deep. It was from a plant of unknown age growing in the field at Manhattan, Kansas. BRUNS (**1**) traced vertical roots of climbing milkweed plants of unknown age growing in the field near Canton, Kansas, to a depth of $6\frac{1}{2}$ feet. None extended below that depth, although roots of field bindweed, *Convovulus arvensis*, immediately adjacent were excavated to a depth of 12 feet without reaching the tip. The compacted soil layers at depths of 26 and 32 inches at the site of the study here reported may have influenced vertical root penetration.

BRUNS (**1**) did not find permanent lateral roots on the plants of climbing milkweed he studied. He believed that permanent lateral roots, if present, were located so near the surface that they were destroyed by a plowing 6 inches in depth that had been made on the area. HITCHCOCK and CLOTHIER (**7**) used permanent lateral roots from an average depth of 10 inches below the surface for regeneration studies. These lateral roots were obtained from field-grown plants of unknown age. In some of the plants observed in the study reported here all the permanent lateral roots of the first order were located in the surface 6 inches of the soil (fig. 4); in other plants many were below that depth (fig. 6).

Research to determine why certain of the branch roots develop more extensively than others and become permanent lateral roots has not been undertaken. It has been conjectured by KENNEDY and CRAFTS (**9**) for field bindweed, and by FRAZIER (**3**, **4**, **5**) for hoary cress, Russian knapweed, and dogbane, that these variations result from differences in the supplies of soil moisture and plant nutrients.

The nature of development and role of the permanent lateral roots of the first order of climbing milkweed are the same as found for dogbane by FRAZIER (**5**). In both plants these roots grow approximately horizontally if variations in slope, soil texture, and moisture supply are taken into consideration. In no instance were the growing tips of these

roots observed to turn downward and thus become vertical roots, as described for field bindweed, hoary cress, and Russian knapweed (**2, 3, 4**).

HITCHCOCK and CLOTHIER (**7**) in 1898 observed in a general way the nature and extent of the horizontal growth of the underground lateral axes of climbing milkweed and recognized these axes as roots rather than as stems. They observed, as was confirmed in the present study, that there was no regularity in the position of the root-borne buds on the lateral roots, but apparently they did not realize the nature of the secondary vertical roots and their relation to the permanent laterals.

The rhizome of climbing milkweed, especially the tip, is fragile and easily damaged. It grows readily through a moist friable soil but has difficulty emerging in a dry soil that has formed a crust. The young rhizome is quite small in diameter near its place of origin (fig. 5), but once it has formed a shoot it may increase rapidly, particularly in a loose soil, to become a much-thickened structure (fig. 6*N*). In striking contrast to field bindweed, which under favorable conditions of growth forms a number of new rhizomes in 5–8 days after the destruction of the existing rhizome, climbing milkweed usually forms but one rhizome for each one destroyed, and emergence requires 14–22 days—even under favorable conditions.

Summary

1. Plants of climbing milkweed, grown from seed on a typical upland loam soil at Manhattan, Kansas, under known temperature and precipitation conditions and not subject to competition, were studied at various ages, from the seedling stage through 25 weeks of growth, to determine the nature and rate of development.

2. The root system of well-established plants consisted of the original root (primary vertical) and one to many permanent laterals which continued to grow horizontally and on which arose roots that grew either downward directly, or did so after short horizontal growth, to become secondary vertical roots.

3. The plants spread horizontally by means of the permanent lateral roots. The permanent laterals of the first order arose on the primary vertical root. Branch lateral roots (laterals of the second order) arose on the permanent laterals of the first order. In a similar manner permanent laterals of the third order arose on those of the second, and those of the fourth order arose on those of the third, etc. Concurrent studies indicated that injury or too severe competition prevents extensive horizontal growth of the laterals of any order.

4. The plants spread radially 9 feet in undisturbed soil (10 feet where the last 4 feet penetrated soil which had been excavated and replaced two years earlier) and reached a depth of $3\frac{1}{2}$ feet in a growing season of 25 weeks.

5. The source of shoot development, other than that arising from the plumule, was from root-borne buds which produced shoots directly (if at the ground line), or rhizomes (if below ground) which in turn gave rise to leafy shoots. There was no regularity in the location of these adventitious buds on the permanent lateral roots. None of these buds was observed on secondary vertical roots. The shoot development of old plants was wholly from root-borne buds.

6. The type of development is similar to that of dogbane, and their common type of development exhibits certain similarities to, and certain differences from, the type common to field bindweed, hoary cress, and Russian knap-

weed. The horizontal spread in both types is by means of permanent lateral roots, but in the dogbane-climbing milkweed type the lateral roots continue to grow horizontally and do not bend downward to produce vertical roots (secondary verticals) as they do in the other type. In the dogbane-climbing milkweed type, secondary vertical roots arise on the permanent lateral roots. The development of shoots appears to be the same in both types. Field bindweed and climbing milkweed were the only two of the five species to flower and fruit during the first growing season.

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SECOND-YEAR DEVELOPMENT OF ROOT SYSTEM OF APOCYNUM CANNABINUM

The nature and rate of development of the root system of this weed, commonly known as dogbane, reported for the first growing season (28 weeks of growth) (BOT. GAZ. **105**:463–470. 1944), have now been studied through the second growing season. The soil profile was further described to a depth of 14 feet by Dr. J. C. HIDE of the Kansas Agricultural Experiment Station. The profile description for the 7–14-foot interval was similar in texture and structure to the 5–7-foot interval described in the article cited, except that it became slightly more friable with depth. The plant most extensively developed in 77 weeks following seedling emergence on May 17, 1943 (from a planting made May 3, 1943), was excavated November 6, 1944. In the two growing seasons its primary vertical root attained a maximum vertical penetration of 13 feet, 8 inches, the deepest secondary vertical root 7 feet, 9 inches, and the most extensively developed permanent lateral root of the first order reached 19 feet, 6 inches, in length. The first flowering of this plant occurred May 25, 1944, and many fruits were matured that year (1944).—JOHN C. FRAZIER, *Contribution no. 468; Department of Botany, Kansas Agricultural Experiment Station, Manhattan, Kansas.*

INVERSE CORRELATION BETWEEN RUBBER HYDROCARBON AND A CRYSTALLINE FRACTION ISOLATED FROM LATEX OF CRYPTOSTEGIA GRANDIFLORA

WILLIAM S. STEWART[1] AND RICHARD W. HUMMER[2]

Introduction

A crystalline fraction has been isolated from dried latex of *Cryptostegia grandiflora*. Available data indicate that there is an inverse correlation between this crystalline fraction and the rubber hydrocarbon in the latex. This relation may afford an opening wedge into the problem of rubber formation in plants.

C. grandiflora, a member of the Asclepiadaceae, is one of several plants being studied as an emergency source of rubber. The plant is either a large shrub or a vine, depending on available support for vining. In addition to foliage branches, it produces relatively long, rapidly growing, mostly unbranched leaders which tend to twine around any available support. These leaders, or whips, as they sometimes are called, usually originate from lateral buds along the thicker main stems which form the body of the shrub. Their diameter near the tip is 2–3 mm., widening to 15–20 mm. near the base. For nearly one-third of their length from the tip they are either completely leafless or the small unexpanded leaves are adnate to the whip. In this region the whips usually are succulent, very slightly lignified, and have a large proportion of pith and cortex, whereas the lower part of the whip is woody, lignified, and with proportionally little pith. A more complete description of the plant is given by POLHAMUS *et al.* (4). Failing to twine on a support, the whip may grow to a length of 12–15 feet before bending downward from its own weight. As the whip continues to grow and bend, short, leafy, lateral branches are produced and it becomes increasingly woody. The weight of these branches in turn contributes to the further bending of the whip down within the body of the shrub. New whips, which often originate from old foliated ones, are continually produced and replace the older ones. Cutting off the tips of the whips before they bend downward and collecting the exuding latex has been one suggested method of utilizing the plant as an emergency source of rubber. This general procedure, called "whip bleeding," was the manner in which most of the latex samples used in this investigation were collected.

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Method

During the course of yield determinations by whip bleeding from March, 1943, through July, 1944, 157 latex samples were collected. These were dried to constant weight in a thermostatically controlled oven at 60° C., instead of being coagulated as usual by some such agent as alcohol or water. For the studies reported here, the importance of drying the latex instead of using a coagulating agent cannot be overemphasized. In common practice, after the use of a latex-coagulating agent, the serum is usually discarded and only the coagulate analyzed. While this may be satisfactory for quality determinations of the coagulate, it yields physiological information of limited value, since a portion

of the latex is discarded with no further consideration.

The samples represented collections of extremely wide diversity. They were secured from the vicinity of Brownsville, Texas, and Ciudad Victoria, Tamaulipas, Mexico. Some of the collections were composite yields of latex from single whips on individual plants bled four to seventeen times consecutively; others were pooled yields from various amounts of successive bleedings of 20 to 50 plants. Some of the samples were from plants 7 months old; others were from plants estimated to be 15 years old. In most collections the whips were of the type just described, although a few samples were from foliage branches of small diameter ($\frac{1}{4}$ inch or less), very old bent-down whips, thick ($1\frac{1}{2}$ inch diameter) basal foliage stems, and seed pods. All collections were from the above-ground portion of the plants. Because of the extremely wide diversity of the collections, the whip internode in which the bleeding was made, the number of previous bleedings of the whip, the time interval between bleedings, the time of day of the bleeding, the season of the year, and the amount of whip cut away per bleeding were factors varying from sample to sample.

The oven-dried latex samples were analyzed for rubber hydrocarbons by a bromination technique and for insolubles by a direct gravimetric determination. These methods were kindly supplied by private communication from Dr. C. O. Willits of the Eastern Regional Research Laboratory, and are as follows.

Accurately weighed samples of the dried latex, sometimes called "total latex solids," are cut into small pieces, placed in tared centrifuge tubes, and treated with benzene containing 1% trichloroacetic acid. After standing for 3 days, with occasional stirring, insoluble substances are separated by centrifuging and washing with benzene. The benzene solution and washings are made to volume with benzene, and aliquots are brominated using a solution of bromine and iodine in carbon tetrachloride. The "rubber tetrabromide" so formed is precipitated with 95% ethyl alcohol, filtered, dried, and weighed. The percentage of rubber in the sample is calculated by multiplying the weight of rubber tetrabromide by the empirical factor of 0.285.

The substances insoluble in benzene which remain in the centrifuge tubes are thoroughly extracted several times with acetone. The insolubles still remaining are separated by centrifuging, dried at 60° C., and weighed. Insolubles prepared by this method—using trichloroacetic acid in the benzene extraction (1)—have been shown to be completely free of rubber hydrocarbons.

By these methods, "insolubles" in latex total solids are defined as substances which are insoluble in either acetone or benzene, and "rubber hydrocarbons" are defined as benzene-soluble substances which form bromides insoluble in 95% ethyl alcohol. The insolubles and rubber hydrocarbons are each directly determined, percentages being based on original weight of latex sample dried at 60° C.

Results and discussion

Analyses of the diverse assortment of latex samples indicated that they varied in such a way that the percentage of rubber hydrocarbons was, without exception, inversely correlated with the percentage of insolubles (fig. 1). The correlation coefficient, significant at the 1% level, was −0.9371. Apparently this inverse correlation was a linear relationship. From these analyses, using the

method of least squares (5) for the computation of regression averages, it was possible to calculate the equation: $R\% = 77.8 - 0.809\, I\%$, where R = rubber hydrocarbons and I = insolubles. The regression coefficient, significant at the 1% level, is −0.809. This equation defines the regression line *RI* (fig. 1) which has intercepts on the rubber hydrocarbons and insolubles axes of 77.8 and 96.2, respectively, and is calculated from 149 analyses. These are theoretical limits based on extrapolation and are subject to experimental error. The standard error of the estimate was 2.6%, which represents graphically an average of the vertical distances of the dots from *RI*. Table 1 gives the analyses of twenty-five of the 149 samples represented in figure 1.

Analyses of twenty-three additional samples of latex dried at 60° C. were made by a direct extraction method described by HALL and GOODSPEED (2). The determined percentages of rubber hydrocarbons and insolubles for each sample were located on the calculated *RI* regression line. These analyses, however, were not used in computation of the regression line, nor are they shown in figure 1, since by this method of analysis the insolubles are determined indirectly. Latex samples of average resin content, analyzed by this method, might appear to have—within the limits of standard error of the regression line *RI*—rubber hydrocarbons and insolubles inversely correlated, solely because of incomplete extraction of rubber hydrocarbons. For this reason an inverse correlation based on analyses by this method may or may not be correct, depending on the completeness of the extractions.

To concentrate the substances present in the insolubles which caused the inverse correlation with rubber hydrocarbons, a survey was made to discover solvents which could extract them from the "insolubles." It was found that they were dissolved by water, by 95% ethyl alcohol, and by absolute ethanol. Further trials indicated that if the insolubles were dried to a constant weight at 100° C. before extraction with ethanol, an additional separation of this extract (*E*) into two portions was possible by a fractional precipitation technique. The separation

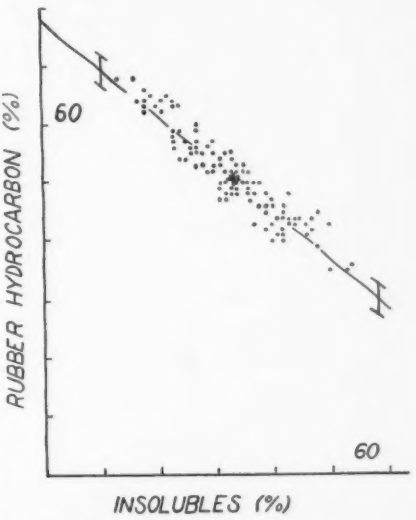

FIG. 1.—Inverse correlation between percentage of rubber hydrocarbons and percentage of insolubles in dried latex of *C. grandiflora*. Regression line, with standard error, calculated from 149 latex analyses. Cross indicates average point.

of latex insolubles into a crystalline fraction inversely correlated with rubber hydrocarbons and noncorrelated fractions is shown in the following schematic representation. Letters in parentheses identify the fraction with the corresponding discussion in the text. The first fraction was obtained by adding petroleum ether to the ethanol until an oily, brown, flocculent precipitate formed (*P*). Failing to form a precipitate on addition of a volume of petroleum

ether equal to that of the ethanol, the solution (*EE*) was partially evaporated at 100° C. until a precipitate formed. In some cases it was necessary—even after this evaporation—to add more petroleum ether to change the solubility relationships in the solution so that a precipitate would form. After quantitatively separating the precipitate from the solution, this same treatment was repeated on the filtrate several times until no further precipitate formed. The second fraction

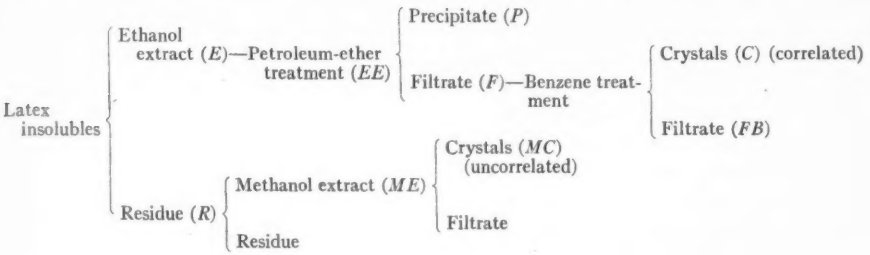

TABLE 1

NUMERICAL DATA FOR TWENTY-FIVE OF THE 149 CRYPTOSTEGIA GRANDIFLORA LATEX TOTAL SOLIDS ANALYSES SHOWN GRAPHICALLY IN FIGURE 1. LATEX OBTAINED BY WHIP BLEEDING UNLESS OTHERWISE INDICATED

Sample no.	Latex source		Collection date	Latex analyses (%)		
	Bleeding	Other data		Rubber hydrocarbons	Insolubles	Undetermined (remainder)
106	2nd	Whips defoliated by hand	2/25/44	35	53	12
59	8th	Fifteenth internode	8/28/43	36	54	10
121	2nd	Entire plant defoliated	2/25/44	37	45	18
37	16th	At 24-hr. intervals	8/29/43	39	48	13
99	7th	Entire plant defoliated	2/5/44	40	40	20
116	1st	Whips 2–3 ft. long	3/9/44	41	42	17
135	1st	Plants in 5-gal. containers	6/2/44	42	42	16
159	4th	At 24-hr. intervals, 6:00 P.M.	3/23/44	43	37	20
169	5th	Plants in 5-gal. containers	6/7/44	44	41	15
113	18th	At 48-hr. intervals	3/24/44	45	39	16
146	1st	2:00 P.M.	3/20/44	46	38	16
11	10th	At 72-hr. intervals	9/10/43	47	36	17
26	6th	At 24-hr. intervals	8/19/43	48	34	18
148	2nd	At 24-hr. intervals, 6:30 A.M.	3/21/44	49	33	18
23	4th	At 24-hr. intervals	8/17/43	50	35	15
151	2nd	At 24-hr. intervals, 6:00 P.M.	3/21/44	51	34	15
131	1st	Sixth internode	5/11/44	52	32	16
2	2nd	Eighth internode	8/15/43	53	34	13
145	1st	Third internode, whips 10 ft. long	6/15/44	54	31	15
144	1st	Third internode, whips 6 ft. long	6/15/44	56	27	17
28	6th	At 24-hr. intervals	8/19/43	58	23	19
166	.....	Seed pods, nearly mature	3/12/44	59	23	18
101	8th	At 48-hr. intervals	8/28/43	63	18	19
109	14th	At 48-hr. intervals, two bleedings per internode	9/9/43	65	19	16
110	14th	At 48-hr. intervals, one bleeding per internode	9/9/43	68	13	19

was then obtained by adding benzene to the filtrate (*F*) until the formation of crystalline platelets began (*C*). Here, as with the addition of petroleum ether, if no crystallization began after the addition of a volume of benzene equal to that of the filtrate, it was necessary to evaporate the solution until crystallization began and in some cases to add more benzene. These manipulations were repeated until no further crystal formation was obtained. Evaporation of the remaining filtrate (*FB*) yielded a residue which was less than 1% of the original sample. This was considered negligible.

The ethanol extract (*E*) of insolubles from latex samples low in rubber hydrocarbons often was dark reddish brown, while from those high in rubber hydrocarbons it was straw-yellow color. After complete precipitation of the brown precipitate (*P*) by the addition of petroleum ether, the filtrate (*F*) was usually light yellow. This color change was indicative of the completion of precipitation. If the precipitation was not complete, crystals (*C*) contaminated with a slight amount of brown color resulted. This was removed by redissolving the crystals in a small volume of hot ethanol, adding powdered activated charcoal to adsorb the brown color, filtering, and then repeating the crystallization procedure. A loss of about 40% usually occurred from this treatment, thus making it unsatisfactory for a quantitative determination of the crystals.

Identification of the crystals (*C*) from the ethanol extract is not yet complete, but a few properties have been determined. They are white, slightly bitter-tasting, and strongly birefringent. They begin to char at 180° C. and melt between 234° and 236° C. Crystals isolated from the serum of auto-coagulated latex of *C. grandiflora* have been reported to have a bitter taste and to melt at 236–7° C. (3), and so they may be the same as those described here.

To determine the extent to which either the flocculent precipitate (*P*) or the crystalline fraction (*C*) might be inversely correlated with rubber hydrocarbons, eight latex samples of known composition were selected. Aliquots of these were re-analyzed to check the previous determinations. These samples varied in percentage of rubber hydrocarbons from 38 to 62 and in percentage of insolubles from 47 to 19. Their selection thus represented a relatively wide distribution along the rubber hydrocarbons–insolubles regression line. The insolubles obtained by the analytical method from these samples were extracted by using four portions of absolute ethanol with stirring. After extraction, the residue was washed with an additional small portion of absolute ethanol and the extracts and washings combined for each sample. These were then separated into crystalline (*C*) and noncrystalline (*P*) fractions by the technique described. The crystalline fraction was inversely correlated with rubber hydrocarbons, but the noncrystalline portion was not correlated (fig. 2). The coefficients of correlation for rubber hydrocarbons and crystals, and for rubber hydrocarbons and noncrystalline portion, were −0.9056 and −0.5753, respectively. The correlation coefficient for crystals was significant at 1%, but that for the noncrystalline portion lacked significance at 5%. The equations with standard error of the estimate, for the regression of percentage rubber hydrocarbons on percentage of insolubles, ethanol extract (*E*), and crystals (*C*), as given in figure 2, are: $R = 75.5–0.780\,I$, $\pm$ 2.6; $R = 70.0–1.025\,E$, $\pm$ 3.2; and $R = 68.8–1.218\,C$, $\pm$ 3.8, respectively. The equa-

tion for the regression of percentage precipitate (P) on percentage rubber hydrocarbons is: $R = 86.4–11.4\ P, \pm 1.1$. This experiment was repeated on three additional latex samples with similar results.

While the crystalline fraction appears to account for the major portion of the observed inverse correlation between rubber hydrocarbons and insolubles,

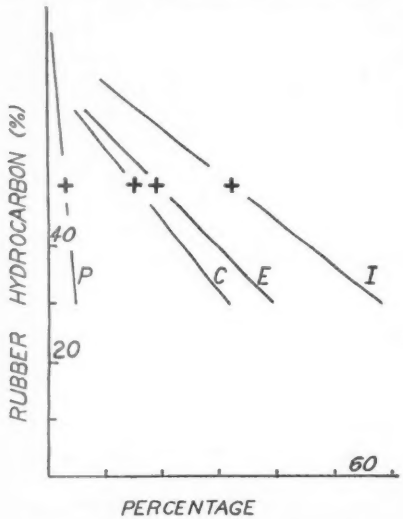

FIG. 2.—Inverse correlation in dried latex of *C. grandiflora* between percentage of rubber hydrocarbons and percentage of: insolubles, regression line *I;* ethanol extract of insolubles, line *E;* and crystalline fraction from ethanol extract, line *C*. Crosses indicate average points in each case. Correlation coefficients for these regressions are −0.9557, −0.9337, and −0.9056, respectively (significant at 1%). Precipitate from ethanol extract (line *P*) not significantly inversely correlated with rubber hydrocarbons.

whether or not it alone accounts for the entire correlation is not known—since an inverse correlation was observed between rubber hydrocarbons and the insolubles residue (R) left after ethanol extraction. The correlation coefficient was −0.7537, significant at 5%. The regression of rubber hydrocarbons on this residue is $73.6–1.779\ IR$, where IR equals the insolubles not extracted by ethanol. Standard error of the estimate is ± 5.09. The regression coefficient is also significant at 5%.

Since the correlation between rubber hydrocarbons and insolubles residue (R) could have resulted from incomplete ethanol extraction of the insolubles, more complete extractions were made by shaking the insolubles overnight in ethanol, following three 10-minute extractions with hot ethanol. These were carried out on the insolubles from three large lots of latex which were being used to prepare a quantity of crystals. The overnight shaking extraction was repeated two additional times on new aliquots of insolubles from the same three lots, which had percentages of rubber hydrocarbons and insolubles of 37, 33; 45, 24; and 56, 12; respectively. The crystalline fraction (C) was obtained from the extract by the fractional precipitation technique described. As in the previous experiment, the crystalline fraction (C) accounted for the correlation of the extract (E), but in every trial the extract still apparently failed to contain all the correlated portion of the insolubles. The insolubles residue (R) remaining after ethanol extraction still had a very slight but significant inverse correlation with rubber hydrocarbons. Owing to mechanical difficulties, because of the large size of the lots, recovery of the insolubles from the oven-dried latex was incomplete. This located the regression line of rubber hydrocarbons on latex insolubles (fig. 3) slightly toward the left of the original line given in figure 1. The slope of this line is the same as that of the original, however, since the determinations of rubber hydrocarbons were not changed by the size of the lots.

Further investigation of this appar-

ently correlated residue (*R*) indicated that it was possible to separate it further by extraction with absolute methanol. From duplicate methanol extractions of the residue (*R*) from the three lots of latex, it was found that this extract (*ME*) was not correlated with rubber hydrocarbons. After extraction, crystal formation was readily induced by reducing the volume of the extract. These crystals (*MC*), like the methanol extract (*ME*), were not correlated with rubber hydrocarbons. Their properties and identity are being determined. It was clear, however, that these crystals differed from those obtained by the ethanol extraction, since these lacked birefringence. They were readily soluble in methanol and also very slightly soluble in ethanol. Because of their ethanol solubility, the amount of ethanol extract (*E*) from insolubles will depend in part on the amount of these noncorrelated crystals (*MC*) present in the insolubles.

A further attempt was made to ethanol-extract all the correlated substances from the insolubles by using micro-Soxhlet extractors. Three insolubles samples of 200 mg. each were extracted 48 hours. The extraction was complete, as indicated by constant weight of the residue during the final 12 hours. During the extraction, crystals precipitated from the ethanol. These crystals lacked birefringence, were very readily soluble in methanol, and appeared to be the same as those just described (*MC*). They amounted to 12.0, 2.9, and 5.4 mg. from the three latex samples which had 56, 45, and 37% rubber hydrocarbons, respectively. In this way the ethanol extract (*E*) of insolubles from samples highest in rubber hydrocarbons was increased by this noncorrelated portion over those from lower percentages of rubber hydrocarbons. Since some of these noncorrelated crystals (*MC*) probably also remained in the ethanol solution, when considered together with those which precipitated out, they could probably account for the convergence of the two regression lines—rubber hydrocarbons on insolubles and rubber hydrocarbons on extract—at the high percentage levels of rubber hydrocarbons (figs. 2, 3).

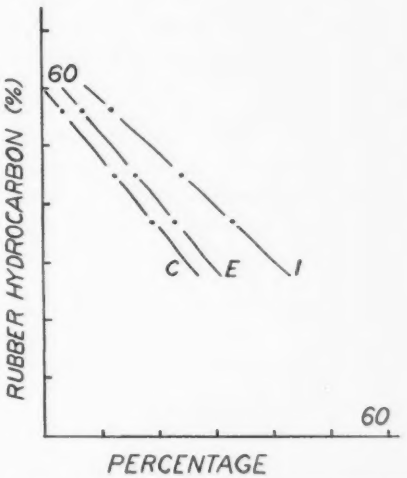

FIG. 3.—Inverse correlation in dried latex between percentage rubber hydrocarbons and percentage of insolubles (*I*); ethanol extract of insolubles (*E*); and crystalline fraction from ethanol extract (*C*).

Methods are being investigated for more completely separating the two crystalline fractions (*C* and *MC*) found in the insolubles. While it seems possible that the crystalline fraction (*C*) from the ethanol extract may be the only one in the latex inversely correlated with rubber hydrocarbons, conclusive proof will await the development of its more selective isolation from the insolubles.

When fresh latex is dried on a sheet of glass, both the correlated ethanol fraction crystals (*C*) and the apparently

noncorrelated "methanol" crystals (*MC*) are visible. The former may be seen readily with the naked eye, but the latter are usually apparent only on microscopic examination. That the "ethanol" crystals (*C*) are readily visible in latex dried in this fashion is not surprising, since they constitute 19% of the total latex solids from latex which has 37% rubber hydrocarbons.

The significance of the inverse correlation between rubber hydrocarbons and the crystalline fraction (*C*) extracted from the insolubles by ethanol is not known. The crystalline substance might be directly converted into rubber hydrocarbons within the plant and so be considered a "precursor" of rubber hydrocarbons; but it is equally possible that it might be a substance very indirectly related to rubber hydrocarbons. For example, it might be derived from the same source of materials as rubber hydrocarbons, and so in effect be indirectly related to them by competition for its origin. Identification of the crystals and physiological experiments in progress may indicate the role this substance has in rubber formation and latex physiology.

Summary

1. Analysis of 157 samples of dried latex from above-ground portions of *Cryptostegia grandiflora* indicated the existence of a linear inverse correlation between the percentage of rubber hydrocarbons and the insolubles.

2. By extraction of the latex "insolubles" with absolute ethanol and fractional precipitation of the extract, an unidentified crystalline fraction was obtained which was inversely correlated with rubber hydrocarbons. It is possible that this crystalline fraction is the only one in the insolubles and latex inversely correlated with the rubber hydrocarbons.

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RUBBER CONTENT, STEM ANATOMY, AND SEED PRODUCTION AS RELATED TO RATE OF VEGETATIVE GROWTH IN GUAYULE

J. W. MITCHELL, A. GERALDINE WHITING, AND H. M. BENEDICT[1]

Introduction

LLOYD (4) observed that, under natural conditions, rubber accumulated most readily in plants of guayule (*Parthenium argentatum* Gray) during those periods of the year least favorable to vegetative growth. BONNER (3) reported that, under experimental conditions, guayule plants attained a higher concentration of rubber in their stems and roots when their vegetative growth was retarded to some extent by subjecting them to night temperatures of 35°–50° F. than when they were subjected to temperature conditions that favored continuous growth. These results suggest that rubber storage in guayule occurs mainly during the winter season, when the rate of vegetative growth is at a minimum.

In the present experiments, plants were grown which differed widely as to succulence and amount of vegetative growth produced during the summer growing season. These differences were secured by varying the amount of nitrogen, and—in some instances—the amount of calcium and nitrogen, supplied to the plants. The plants were studied in regard to (*a*) their relative efficiency in producing rubber, (*b*) the anatomical characteristics of their stems with respect to their capacity for rubber storage, and (*c*) the number and quality of seeds produced.

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Methods

Seedlings (strain 593) that had been grown for one season under field conditions at an Emergency Rubber Project nursery near Salinas, California, were selected for size and uniformity. They were planted in a mixture of coarse sand and fine gravel contained in 3-gallon glazed earthenware crocks provided with adequate drainage. Two experiments are reported here.

In preparing a basic nutrient solution for the first experiment, sufficient $CaCl_2$ was added to tap water to make a concentration of 17.5 milliequivalents of calcium per liter and sufficient KH_2PO_4 and $MgSO_4$ to make a concentration of 2.6 me. of the respective ions per liter of nutrient. Sufficient ferric citrate, manganese chloride, cupric chloride, zinc chloride, and boric acid were added to make 0.3, 0.25, 1.0, 1.0, and 1.5 p.p.m. of Fe, Mn, Cu, Zn, and B, respectively. Treatments consisted of five nutrient solutions made by adding sufficient $NaNO_3$ to the basic solution to make 1.4, 3.6, 7.2, 14.4, and 28.8 me. of NO_3 ion, respectively.

The pots, each containing one plant, were arranged on a gravel bed in successive rows of four plants each. The experimental design consisted of randomized blocks with sixteen plants per treatment and four replications of each treatment (*fig. 1*). Approximately 400 ml. of nutrient solution was applied to each pot three times weekly, an amount sufficient to flush the gravel thoroughly. Tap water was used to maintain moisture supply between nutrient treatments. The plants

were grown out-of-doors from July to October, 1943, then arranged in the same order in a greenhouse, where temperatures of 35°–45° F. were maintained during the night and 60°–70° F. during the day. In February, 1944, all plants were harvested, weight and anatomical measurements were made, and the rubber and resin contents of the roots and stems were determined by the SPENCE and CALDWELL method (7).

In the second experiment, selected plants from the same shipment of seedlings were grown in a similar manner. The basic nutrient used contained 2.6 me. of K, Mg, H_2PO_4, and SO_4 ions per liter and the same concentrations of microelements as previously described. The concentration of calcium and nitrate ions was varied from 1.8 to 7.2, 14.4, and 21.5 me. per liter by adding desired amounts of $Ca(NO_3)_2$.

The plants were grown out-of-doors, arranged in rows of five plants each. Treatments were applied as randomized blocks with eight variations of nutrient and five replications, as previously described. The experiment was started during the first week of June, 1943. Plants of treatments 1, 2, 3, and 4 received nutrient containing 1.8, 7.2, 14.4, and 21.5 me. of calcium and nitrate ions per liter, respectively, the treatments being continued until the plants were harvested in February, 1944. Plants of treatment 5 received 1.8 me. of calcium and nitrate ions during approximately the first half of the growing season (July 21, 1943), then 21.5 me. during the remainder of the growing season (which ended the latter part of September) and winter dormancy period. Plants of treatment 6 received 7.2 me. and then were changed in a similar way to 14.4 me. per liter; those of treatment 7 received 14.4 me. and later 7.2 me.; while those of treatment 8 were supplied with nutrient containing 21.5 me. of calcium and nitrate ions per liter for the first half of the growing season and then shifted (on July 21, 1943) to a solution that contained 1.8 me. In this way the growth of some plants was limited by nu-

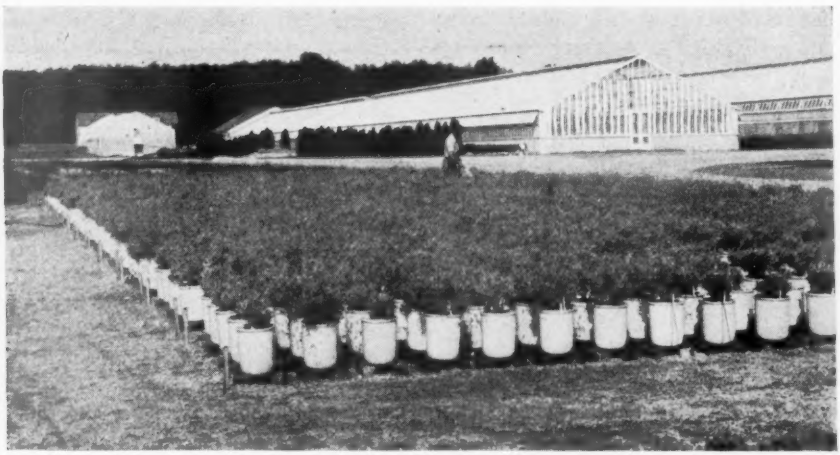

FIG. 1.—Field arrangement of guayule plants and greenhouses in which the plants were grown during the winter months under controlled temperatures.

trient treatments during the entire experiment, while others supplied with adequate nutrients grew vigorously during the summer and fall, their growth being checked by low temperatures during the winter months. Some were made to grow slowly during the first part of the summer and then rapidly during the remainder of the growing season, while others grew rapidly at first and then more slowly during the latter part of the growing season. All plants were moved to a greenhouse in October and subjected to the same light and temperature conditions throughout the winter dormancy period. Nutrient treatments begun during summer were continued unchanged throughout the fall and winter seasons.

Seeds were collected weekly during four periods: from June 1 to July 15, July 15 to August 9, August 9 to September 1, and September 1 to September 16. They were cleaned and stored at room temperature during the following winter. Later they were sorted for size, and tested for germination as described previously (**6**).

At the time of final harvest (February, 1944), material for a limited anatomical survey was collected. Of those plants grown with variation in the calcium and nitrogen supply, samples were selected from each of three plants from each of four levels of nutrition: 1.8, 21.5, 1.8 changing to 21.5, and 21.5 changing to 1.8 me. of nitrate ion per liter, with the accompanying changes in calcium. One series of samples consisted of segments 1 cm. in length taken from the main stem of each plant in the region just above the cotyledonary node. The extent of topping previous to transplanting and the consequent low branching of the plant caused these segments to be highly irregular in form and difficult for anatomical measurements. Accordingly, a second series of samples from each plant was secured from the base of a major branch representative in size for the general growth of the plant. Of those plants grown with varying amounts of nitrogen, similar samples were selected from the base of each of two average branches of three plants each from the five levels of nitrogen nutrition. The methods used in preparing slides and determining cross-sectional areas of wood, bark, and fiber tissue in the bark have been described previously (**8**).

The term "significant" is used in this paper to mean that differences between means were significantly different by odds of 19:1.

Results

RESPONSES TO VARIATIONS IN NITROGEN SUPPLY.—An increase in nitrogen supply from 1.4 to 14.4 me. per liter of nutrient resulted in marked increase in vegetative growth, as reflected in the dry weight of stem and roots; further increase in nitrogen supply up to 28.8 me. did not significantly increase the growth of stems and roots as measured by their dry weight (table 1). In contrast, the percentage of rubber in the stems and roots was not influenced by these extreme variations in the amount of nitrogen supplied to the plant. As a result, the total rubber produced per plant paralleled the amount of its vegetative growth.

Increasing the nitrogen supply from 1.4 to 14.4 me. was associated with a significant increase in the cross-sectional area of the branches (table 2); increase above this amount, up to 28.8 me., resulted in no further significant increase in diameter. The percentage of bark in stems of plants that were stunted owing to the limited amounts of nitrogen supplied was greater than that of plants which received increased amounts of ni-

trogen and grew vigorously. However, this difference was not wholly due to the nutrient treatments, but was largely due to inherent differences in proportions of tissues in small and large stems. This

TABLE 1

EFFECT OF VARYING THE AMOUNT OF NITRATE ION IN THE NUTRIENT ON GROWTH AND AMOUNT OF RUBBER PRODUCED BY GUAYULE. EIGHT MONTHS AFTER TRANSPLANTING

Nitrogen per liter of nutrient (me.)	Average fresh weight per plant (gm.)	Average dry weight of stems and roots (gm.)	Average percentage rubber in stems and roots (%)	Average rubber per plant (gm.)
1.4.....	38.7	9.1	4.99	0.45
3.6.....	66.4	14.4	4.99	0.72
7.2.....	105.1	21.8	4.99	1.09
14.4.....	147.7	31.0	5.09	1.58
28.8.....	172.6	35.0	4.77	1.67

"size effect" was observed in an analysis of sections at different distances from the tips of branches of plants grown under similar field conditions. The data showed that changes in total area, from the very young stems of approximately 7 mm.^{2} in cross-sectional area to older stems of 90 mm.^{2}, were associated with a decrease in bark percentage from approximately 65% to 56% of the total cross-sectional area of the stems. Further increase in stem area, up to 200 mm.^{2}, showed only a slight decrease of 1% or 2% in average percentage bark area. The branch areas of the plants supplied with different amounts of nitrogen fall within the sharper portion of this curve. The significant differences in percentage area of bark observed in connection with the nutrient treatments are therefore considered to be due mainly to "size effect." They do not appear to be correlated with rubber concentration of the stems (tables 1, 2).

RESPONSE TO SEASONAL VARIATIONS IN CALCIUM AND NITROGEN SUPPLY.—Very marked increase in vegetative growth resulted when the calcium and nitrogen supply of the plants was increased from 1.8 to 21.5 me. per liter of nutrient (fig. 2; table 3). Greatest growth resulted when plants were supplied with a relatively large amount of calcium nitrate (21.5 me. per liter) during the entire growing season. The percentage of rubber in the stems and roots of plants grown with extremely small amounts of calcium and nitrogen (1.8 me. per liter) was significantly less than that of other plants supplied with larger amounts (7.2–21.5 me. per liter). The over-all production of rubber closely paralleled

TABLE 2

EFFECT OF VARYING THE AMOUNT OF NITRATE ION IN THE NUTRIENT ON RELATIVE PROPORTIONS OF STEM TISSUES, BASED ON COMPARABLE TRANSVERSE SECTIONS FROM BASAL PORTIONS OF REPRESENTATIVE BRANCHES. EIGHT MONTHS AFTER TRANSPLANTING

Nitrogen per liter of nutrient (me.)	Average total stem area (mm.^{2})	Percentage		
		Wood per total stem area	Bark per total stem area	Bark fiber per total bark area
1.4.....	11.1	33.8	66.2	14.5
3.6.....	13.6	31.6	68.4	13.7
7.2.....	23.0	36.2	63.8	13.5
14.4.....	31.8	41.0	59.0	17.3
28.8.....	27.8	40.0	60.0	19.9

the amount of vegetative growth produced by the plants.

The greatest number and best quality of seeds with respect to size and percentage germination were produced by plants that received a relatively large and continuous supply of calcium and nitrogen during the growing season (table 4). Seeds from plants supplied with a rela-

tively small amount of calcium and nitrogen (1.8 me. per liter) were of very low quality, especially those which ripened during July and August, when relatively high temperatures prevailed. In general, seeds of relatively good quality were obtained during the spring and fall, while those collected during the warmest part of the growing season were of low quality, irrespective of the nutrient treatment.

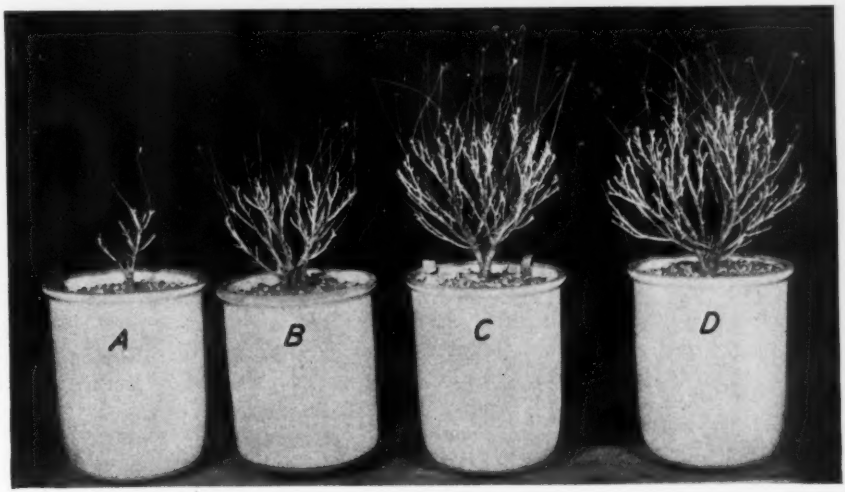

FIG. 2.—Guayule plants grown for 10 months in nutrient cultures containing calcium nitrate: *A*, 1.8 milliequivalents; *B*, 7.2 me.; *C*, 14.4 me.; *D*, 21.5 me. Leaves removed to show size and extent of branching.

TABLE 3

EFFECT OF VARYING THE AMOUNT OF CALCIUM NITRATE IN THE NUTRIENT ON GROWTH AND AMOUNT OF RUBBER PRODUCED BY GUAYULE. NINE MONTHS AFTER TRANSPLANTING. CHANGES IN COMPOSITION OF NUTRIENT IN TREATMENTS 5, 6, 7, AND 8 MADE IN JULY, APPROXIMATELY MIDDLE OF GROWING SEASON

Treatment no.	$Ca(NO_3)_2$ per liter of nutrient (me.)	Average fresh weight per plant (gm.)	Average dry weight of stems and roots (gm.)	Average percentage rubber in stems and roots	Average rubber per plant (gm.)
1	1.8	46	16.0	3.22	0.52
2	7.2	141	43.8	4.51	1.54
3	14.4	212	59.0	4.22	2.49
4	21.5	269	77.8	4.71	3.66
5	1.8 to 21.5	199	48.0	4.61	2.21
6	7.2 to 14.4	194	56.4	4.80	2.71
7	14.4 to 7.2	167	48.3	4.49	2.17
8	21.5 to 1.8	122	38.0	4.63	1.76

Increasing amounts of calcium and nitrogen in the nutrient were associated with great and significant increase in the

total stem and branch areas (table 5). The percentage area of bark of the stem decreased to the extent associated with the "size effect," or changes in the actual area. The increase in the area of wood was correlated with the decrease in the percentage area of bark. There were no significant changes in the fiber in the bark of the stem. In the branches, the percentage area of the bark in plants grown with low amounts of calcium and nitrogen (1.8 me. per liter) was significantly greater than in plants supplied with a greater amount (21.5 me. per liter). The increase in bark area was somewhat greater than could be accounted for by the change associated with size.

TABLE 4

EFFECT OF VARYING THE AMOUNT OF CALCIUM NITRATE IN THE NUTRIENT ON PERCENTAGE GERMINATION OF SEEDS PRODUCED BY GUAYULE. CHANGES IN COMPOSITION OF NUTRIENT IN TREATMENTS 5, 6, 7, AND 8 MADE IN JULY, APPROXIMATELY MIDDLE OF GROWING SEASON

Treatment no.	$Ca(NO_3)_2$ per liter of nutrient (me.)	Percentage germination			
		June 1 to July 15	July 15 to Aug. 9	Aug. 9 to Sept. 1	Sept. 1 to Oct. 16
1	1.8	17.5	1.5	6.0	20.0
2	7.2	20.3	8.0	26.5	27.5
3	14.4	15.0	7.3	37.5	29.8
4	21.5	15.3	6.8	31.7	36.3
5	1.8 to 21.5	19.2	12.3	22.0	26.8
6	7.2 to 14.4	16.8	5.8	29.8	29.5
7	14.4 to 7.2	12.5	6.3	44.5	25.3
8	21.5 to 1.8	12.8	8.5	29.5	22.5

TABLE 5

EFFECT OF VARYING THE AMOUNT OF CALCIUM NITRATE IN NUTRIENT ON RELATIVE PROPORTIONS OF STEM TISSUES, BASED ON COMPARABLE TRANSVERSE SECTIONS FROM BASAL PROPORTIONS OF REPRESENTATIVE BRANCHES. NINE MONTHS AFTER TRANSPLANTING. CHANGES IN NITROGEN CONCENTRATION OF NUTRIENT IN TREATMENTS 4 AND 5 MADE IN JULY, APPROXIMATELY MIDDLE OF GROWING SEASON

Treatment no.	$Ca(NO_3)_2$ per liter of nutrient (me.)	Average total stem area ($mm.^2$)	Percentage		
			Wood per total stem area	Bark per total stem area	Bark fiber per total bark area
1	1.8	12.2	31.2	68.8	24.5
4	21.5	51.2	42.6	57.4	27.0
5	1.8 to 21.5	40.1	39.3	60.7	29.5
8	21.5 to 1.8	34.6	35.6	64.4	29.1

Discussion

In previous experiments, the vegetative growth of guayule plants was con-

trolled by altering their external supply of boron (5) and by subjecting them to various light intensities (6), with the result that decreased vegetative growth during the summer growing season was associated with a decrease in the concentration of rubber in the plant as measured at the end of the following winter dormancy period. In the present experiments, the vegetative growth of guayule was controlled to some extent by controlling the nitrogen, or calcium and nitrogen, supply. Some plants were made to grow vigorously during the entire growing season, while others grew vigorously during the first half of the season but more slowly during the latter part; still others were made to grow slowly at first and then rapidly during the latter part of the summer growing season.

Except for extreme nitrogen and calcium starvation, the percentage of rubber in stems and roots of plants supplied with limited amounts of these elements, or of nitrogen alone, was not significantly different from that of plants grown with an optimum supply. Plants grown with a nutrient deficient in nitrogen showed relatively small growth during the summer, and at the lower nitrogen levels showed extreme symptoms of nitrogen deficiency in the fall, but they attained a rubber concentration during a winter dormancy period equal to that of others which had grown vigorously during the entire summer as the result of adequate nutrient supply. Checking the vegetative growth of plants during either the first or the latter part of the growing season by decreasing the external nitrogen and calcium supply did influence the accumulation during the following winter dormancy period, as indicated by the concentration of rubber in stems and roots. In these experiments, nutrient treatments favorable to maximum growth were likewise favorable to high rubber yields, mainly because of the relatively large size attained by the plants supplied with adequate nutrient, rather than because of the ability of such plants to synthesize more rubber per unit of plant weight.

Since rubber is stored mainly in the bark of guayule, attention has been directed toward its thickness in plants subjected to various environmental conditions. The relative over-all area of the bark and the area of the bark occupied by non-rubber-bearing fiber have been used, with proper consideration of "size effect," as an index to the volume of rubber-storing tissue per unit length of stem. In the present experiments, the diameter of the stems varied in proportion to the amount of nitrogen supplied to the plants. The relative area of bark was essentially constant, except in those treatments supplying very small amounts of nitrogen and calcium, and possibly of nitrogen alone. In previous experiments (5), plants supplied with varying amounts of boron showed marked differences in stem diameters but possessed proportionately constant percentage areas of bark, except in the case of those plants grown with a deficiency of boron. Variation in the intensity of light to which the plants were exposed has also a marked effect on stem diameter but was ineffective in changing the relative proportion of bark and wood (6). LLOYD (4) and ARTSCHWAGER (2) report that the bark in nonirrigated plants was relatively thicker than in irrigated plants. ADDICOTT and PANKHURST (1) found that low—as compared with high—moisture stress increased cambial activity, which resulted in greater areas of both xylem and phloem; and with intermediate stress the development of xylem appeared to have priority over that of

phloem. In general, the results of our anatomical studies indicate that the relative proportions of bark and wood in the stems of guayule plants remain essentially constant, irrespective of relatively wide variations in the nutrient supply—except that conditions of deficiency may result in a slightly increased proportion of bark. ADDICOTT and PANKHURST also concluded that it is apparently difficult to modify the fundamental pattern of development in guayule by the physiological condition of the plant. This general constancy of proportion is not correlated with the total rubber output of the plant, since the latter may be greatly influenced by variation in nutrient supply.

Summary

1. The growth of guayule in gravel cultures was controlled by supplying the plants with nutrient containing various concentrations of calcium and nitrogen ions. Some plants were made to grow vigorously during the entire summer growing season, the growth of others was checked during the entire season, others were made to grow vigorously during the first half of the season and then slowly, while still others grew slowly during the first part of the season and then vigorously during the latter part of the summer. All were subjected to the same light and temperature conditions during the winter dormancy period.

2. The amount of vegetative growth made during the summer season was not related to the plant's ability to synthesize rubber, as based on the percentage found in the stems and roots following the winter dormancy period. Small plants showing nitrogen deficiency symptoms were as efficient with respect to rubber storage as were relatively large plants grown with a much greater nitrogen supply. The total rubber production was directly related to the amount of growth made by the plants during the summer growing season.

3. Seeds of low quality with respect to percentage germination were produced during the warmest period of summer, irrespective of the amount of calcium or nitrogen supplied to the plants. Seeds collected during the spring and fall gave a relatively high percentage germination, and their number and quality were favored by adequate nutrient supply.

4. The relative area of bark of plants grown with varying but not deficient amounts of nitrogen was essentially constant when the inherent differences due to variation in the size of the stem were discounted. A slight increase occurred in the relative thickness of the bark in branches of plants supplied with relatively small amounts of calcium and nitrogen. This increase was not associated with an increase in the percentage rubber in the stems.

The rubber determinations were made by R. L. HOLMES and the statistical analyses by MARY W. SHANOR, of the Special Guayule Project.

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VARIETIES OF KENAF (HIBISCUS CANNABINUS), A BAST FIBER PLANT, IN CUBA

JULIAN C. CRANE[1] AND JULIÁN B. ACUÑA[2]

Introduction

In 1942, seeds from plants of *Hibiscus* were introduced into Cuba from El Salvador under the name of roselle (*Hibiscus sabdariffa* var. *altissima*), in an effort to determine the possibilities of producing this plant as a source of soft fiber for the manufacture of burlap and other articles in which jute (*Corchorus capsularis*) fiber is used. This plant offered a speedy source of material, as the soft fiber obtained from the bast layer or the tissue just under the bark of the stem is ready for harvesting about 90 days after the crop has been planted. Recently, however, the identity of the plant has been established as kenaf (*Hibiscus cannabinus* L.), which—under favorable soil and climatic conditions—is capable of producing more than a ton of dry fiber per acre.

The seed introduced from El Salvador was a heterogeneous mixture containing at least two varieties of this species, as evidenced by the fact that all the plantings in Cuba observed by the writers have been found to contain two morphological types of plants—one having digitate leaves and the other having cordate ones. The majority of the plantings contained about 75% of the digitate type, but in a few instances the proportion was about half digitate to half cordate. The plants grew to an equal height and were alike with respect to growth habit, color of leaves, stems and flowers. But one variety was characterized by having cordate leaves at the bottom of the plant; 3-, 5-, and 7-palmately lobed leaves, respectively, up the middle; and 3-palmately lobed leaves on the top portion. On the other hand, the second variety was characterized by having only cordate leaves throughout the entire length of the stem. This situation was perplexing to local producers as well as to research workers investigating the plant in Latin America.

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Investigation

SPECIES AND VARIETIES

Hibiscus sabdariffa may be distinguished from *H. cannabinus*, according to RAO and SESHADRI (8), by differences in color of the flower petals after they have been picked from the plant. The petals of *H. sabdariffa*, which are pale yellow in color, acquire a red tinge after removal, while those of *H. cannabinus* do not.

According to MCVAUGH (6), these

species also exhibit the following differences in seed characteristics (fig. 1):

H. cannabinus.—"Seeds generally triangular, angles rather acute; surface dull gray with numerous conspicuous yellowish brown raised spots; hilum yellowish brown, relatively small.

H. sabdariffa.—"Seeds generally rounded-hemispherical, angles rounded; surface dull gray without spots; hilum brownish red, relatively large."

FIG. 1.—Seeds of *Hibiscus*. × 2: Those of *H. cannabinus* (top) are generally triangular in shape, with acute angles; those of *H. sabdariffa* (bottom) are generally rounded-hemispherical, with rounded angles. Photographs of this and of succeeding illustrations by JAMES T. MITCHELL, Office of Foreign Agricultural Relations, U.S.D.A.

In addition to these differences in color of withered flower petals and seed characters, there are also noteworthy differences in various floral parts and in the appearance of their stems, as evidenced by the following key and botanical descriptions of HOOKER (3):

"Bracteoles more than 5, free or connate at the base, not adnate to the calyx.

H. cannabinus L.; DC. Prodr. i. 450; annual or perennial, prickly, stem glabrous, lower leaves entire upper lobed, mid-nerve glandular beneath, peduncle very short, bracteoles 7–10 linear, shorter than the calyx, sepals glandular. . . . .

Stem glabrous, prickly. Lower leaves cordate, upper deeply palmately lobed, lobes narrow, serrate; petiole prickly, lower much longer than the blade. Stipules linear, pointed. Peduncles axillary, very short. Sepals bristly, lanceolate, connate below the middle, with a gland at the back of each. Corolla large, spreading, yellow with a crimson centre. Capsule globose, pointed, bristly. Seeds nearly glabrous. . . . .

Bracteoles adnate to the base of the calyx.

H. sabdariffa L.; DC. Prodr. i. 453; annual, glabrous, unarmed, stem purplish, leaves entire or lobed, glandular beneath, peduncles very short, thickened at the summit, bracteoles 8–12 linear, adnate to the base of the calyx, sepals bristly. . . . .

Erect. Leaves polymorphous, midrib glandular beneath; petiole 2 in. Peduncle and calyx accrescent. Sepals deltoid, acuminate, connate below the middle into a purplish fleshy cup. Corolla $2\frac{1}{2}$ in. diam., yellow. Capsule ovoid, pointed, villous, shorter than the calyx. Seeds reniform, sub-glabrous. . . . ."

The differences included in the preceding descriptions are summarized in table 1. According to these identifying characters, the plant material in Cuba evidently corresponds with *Hibiscus cannabinus* and not with *H. sabdariffa*.

Perhaps the most comprehensive botanical investigation conducted on the species *cannabinus* was by HOWARD and HOWARD (5) in India. From the material they collected, the following five varieties comprising eight agricultural types were selected and described:

"1. Variety *simplex*

Type 1.—Stems purple; leaves entire with purple petioles.

2. Variety *viridis*
Type 2.—Stems green; leaves entire with green petioles.
3. Variety *ruber*
Type 3.—Stems red below, greenish above; leaves divided with green petioles.
4. Variety *purpureus*
Stems purple; leaves divided with purple petioles.
Type 4.—Late, stems very tall and slender; leaves with narrow lobes of a diffused purple colour; petals purplish.
Type 5.—Early; stems short and robust; leaves green with broad lobes.

base numerous stout branches which grew parallel to the main stem. They recommended the three types of variety *vulgaris* with their long unbranched stems for fiber production. Investigations in Cuba have shown that there is little difference in the ultimate height of these two varieties and that their tendency for branching is practically identical. Variety *viridis*, planted at four different planting distances, branched only 0.4% more than did *vulgaris*, planted at

TABLE 1

COMPARATIVE DIFFERENCES BETWEEN HIBISCUS CANNABINUS AND H. SABDARIFFA

Plant part	H. cannabinus	H. sabdariffa
Flower:		
Bracteoles	Not adnate to calyx	Adnate to base of calyx
Color of corolla		
Fresh	Pale yellow to sulphur	Pale yellow
Withered	Pale yellow to sulphur	Pale yellow with reddish tinge
Seed:		
Shape	Triangular, angles acute	Rounded-hemispherical, angles rounded
Surface	Dull gray with many yellowish brown, raised spots	Dull gray without spots
Hilum	Yellowish brown, relatively small	Brownish red, relatively large
Stem	Prickly	Unarmed

5. Variety *vulgaris*
Stems green; leaves divided with green petioles.
Type 6.—Plants very early.
Type 7.—Plants late; seedlings with reddish stems.
Type 8.—Plants late; seedlings with green stems."

By comparing the color plates and the descriptions of the material presented by HOWARD and HOWARD (5) with the plant material from Cuba, the writers have been able to identify two varieties of kenaf: *viridis* (type 2) and *vulgaris* (type 8). HOWARD and HOWARD found that variety *viridis* was dwarf in form as compared with the others, and that it had a strong tendency to produce from the the same distances. Likewise, the percentage fiber content was found to be practically the same in the two varieties.

HORST (4), in Java, obtained seed of seven of the types selected by HOWARD and HOWARD and studied the plants grown from this seed in an effort to determine which of these types were grown in Java and the extent of their adaptability to different locations in that country. From his investigation he strongly recommended varieties *viridis* and *vulgaris*. He stated that *viridis* was grown in the eastern part of Sumatra, whereas in western Java the *purpureus* variety (the "red-stemmed" type) was popular. The green variety *vulgaris*, according to

HORST, was the most popular one grown in western Java after the variety *purpureus*. As the seed which was introduced into Cuba from El Salvador was reported to have come originally from commercial plantings in Java, it is logical to assume that the varieties in Cuba are representatives of those grown in Java. Two of these, *viridis* and *vulgaris*, have been identified; the other, *purpureus*, has not been ascertained.

The following description of the two varieties in Cuba is presented for a basis of comparison:

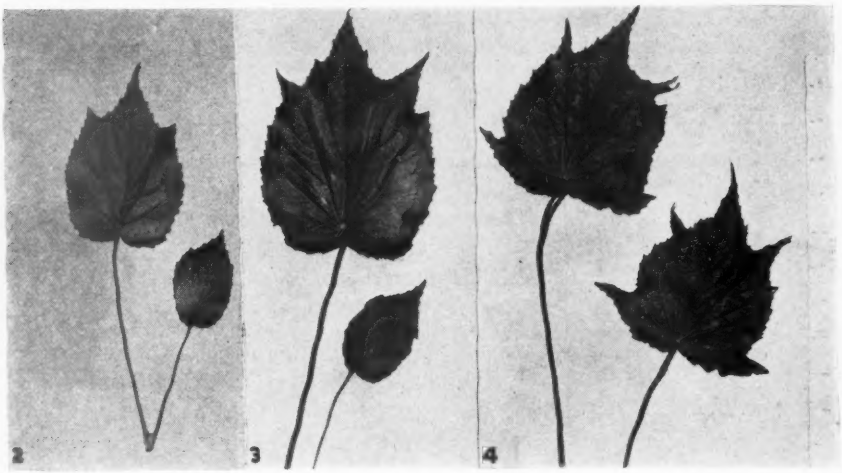
PLANT.—Herbaceous annual of 3–7 months' duration, depending on time of planting.

STEM.—Straight, simple, more or less glabrous but with prickles; 3–14 feet in height, depending on time of planting.

LEAVES.—Cordate and very shallowly lobed with serrated margins (figs. 2–4), mid-vein with one gland beneath near base of blade, petioles generally longer than the blades, with prickles slanting toward the blade; stipules linear and pointed.—*Hibiscus cannabinus* var. *viridus* (type 2).

Basal leaves cordate and not lobed, 3-, 5-, and 7-palmately lobed into narrow serrate parts, respectively, up middle portion and 3-palmately lobed into narrow serrate parts at top of stem (figs. 5–7); mid-vein with one gland beneath near base of blade; petioles generally longer than the blades, with prickles slanting toward the blade; stipules linear and pointed. —*Hibiscus cannabinus* var. *vulgaris* (type 8).

FLOWERS.—Solitary, with short peduncles in axils of leaves; corolla large, spreading, thickened below and thin above, petals pale yellow to sulphur-colored with crimson to purplish center; epicalyx stiff, consisting of 7–8 bracteoles, 10–13 mm. long, which are free above, connate below, and inserted near base of calyx; calyx bristly, lanceolate, 29–31 mm. long, connate below the middle, 5-parted with one large gland near the middle; style rises through staminal column, one in number which terminates in 5 stigmatic branches; carpels 5, joined into 5-locular capsule, each locule containing 4 to 5 seeds; capsule bristly, globose, pointed; seeds glabrous.



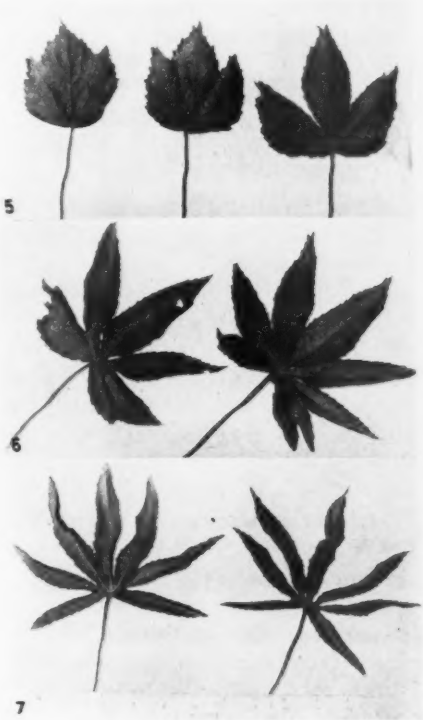
FIGS. 2–4.—*Hibiscus cannabinus* var. *viridis:* Fig. 2, primary with axillary leaf from bottom portion of stem. Fig. 3, same from middle portion. Fig. 4, leaves from adjacent nodes on top portion of plant.

The preceding descriptions indicate that these two varieties are alike in all external characteristics except shape of leaves. USTINOVA (10), however, investigating the percentage of natural cross-pollination in the different varieties,

found that *viridis* was entirely self-pollinated, while in *vulgaris* cross-pollination varied from 2.58 to 39.2% in its different strains. In a previous study (9) she found that irregularities in the structure in the flowers of *vulgaris* were frequent and variable. Although the percentage of staminate and pistillate flowers was not large, nevertheless they did exist. The percentage of undeveloped ovaries in this species was found to vary between 5.5 and 9.5%.

Observations on the two varieties in Cuba have indicated that they are composed possibly of a number of strains, in that there has been some variation in the period of vegetative development among plants. For example, in one particular planting there were a few outstanding plants which were green and had retained all their leaves, while the remainder had dropped most of theirs and seeds had matured. Similar observations have been made by BERLAND (1) in Russia, where he found that *vulgaris* consisted of a number of strains differing by their length of growth period, by their habit of growth and branching, and by some other less important peculiarities connected closely with length of the vegetative period. Strains of the variety *vulgaris* were placed by BERLAND into four groups. The vegetative period of the first group ranged 90–110 days; that of the second, 110–120; the third, 120–130; and the fourth, 130–150 days.

POPOVA (7) showed that the differences in strains of *Hibiscus cannabinus* were in the height of the plant, the thickness of the stem, the color, and in the leaves and flowers. He found strains with large and small seed capsules, and with large and small seeds. He noted that the strains from Central Asia were most frequently mid-season ones, with large capsules and large seeds; whereas the bulk of strains from Persia (representatives of the variety *vulgaris*) were small-capsuled and small-seeded, with a later maturity than that of the strains from Central Asia. POPOVA further noted that the earlier the strain, the closer to the ground the first flowering node was located; in the late strains, however, flowering began on the upper nodes.



FIGS. 5–7.—*Hibiscus cannabinus* var. *vulgaris:* Fig. 5, leaves from adjacent nodes of bottom portion of plant. Fig. 6, 5- and 7-palmately lobed leaves from middle portion of stem. Fig. 7, 7-palmately lobed leaves from adjacent nodes near top portion of stem.

The variations in strains within a particular variety might provide means of improving the plant for fiber purposes by selection in the field for one or two of

the most obviously worthwhile characters, such as height of the first capsule and length of the stem. Some of the characters which an ideal fiber plant should possess are long thin stems, no branches, rapidity of growth, and high fiber percentage with uniform length and strength. As this species is naturally self-pollinated, progress in breeding should be rapid and easy.

Polymorphism of leaves

The accompanying illustrations show pronounced polymorphism of leaves in this species, not only among leaves of different varieties but also among those within the same variety (figs. 3, 5). Cook (2) noted that these variations in leaf forms showed a curious parallel with cotton, a relative of kenaf. He observed that in addition to the entire or very broadly lobed leaves of *viridis* (fig. 2), comparable with those of ordinary Upland varieties of cotton, there were also varieties of kenaf with deeply divided, narrow-lobed leaves, like the so-called "okra" varieties of cotton. Other varieties of kenaf have their leaves parted to the base into narrow digitate segments, a condition also known in some of the tropical varieties of cotton. Further similarity was found, according to Cook, in the fact that *vulgaris* with lobed leaves produced entire leaves at the base of the stalk (fig. 5), as also occurred with the narrow-lobed okra varieties of Upland cotton.

The leaves of the middle and upper part of the stem in *vulgaris* are all deeply lobed (figs. 6, 7), while those of the lower part are without lobes. The transition from one type to the other sometimes occurs between successive nodes (fig. 5), while in other cases this change occupies three or four nodes. A premonition of the change may be found in the larger marginal teeth of the last of the undivided leaves. A more definitely intermediate condition appears when a leaf is divided on one side but only partially so on the other (center leaf in fig. 5). In cases like this there is a marked difference between the two sides of the leaf, so that the change from the entire to the lobed condition is still quite rapid in comparison with the very gradual changes shown in many plants in changing from the large basal or radical leaves to those of the upper part of the stem.

Cook (2) stated that it was difficult to imagine any practical advantage to be gained by the plants in changing the form of the leaves up the stalk, but he advanced the suggestion that different forms possibly might be connected with the fact that there is a difference of function among the internodes of the stalk. As change of leaf form marks the approach of the fruiting condition in such plants as *Hedera helix* and *Ficus repens*, he thought that the divided leaves in kenaf might indicate in advance the internodes that were to produce flowers and fruit.

The writers have not observed this to be true, however, in that flowering in this plant is dependent on photoperiod; and if planted during the season of the year when the days are short, the plants form flower buds before any divided leaves are evident. As this plant is sensitive to photoperiod, the nodes at which flowering takes place are entirely dependent on time of planting and not on change in leaf form. Although the writers have not made a study of this phenomenon, they are of the opinion that this change in leaf form is perhaps the result of some ecological adaptation.

Summary

1. The species of *Hibiscus* being grown in Cuba for the production of soft fiber has been correctly identified as

kenaf (*H. cannabinus*) rather than as roselle (*H. sabdariffa*). Differences in color of withered flower petals and seed characters, as well as unlikeness in various floral parts and in the appearance of stems, are suggested means of distinguishing these closely allied species.

2. Two varieties of kenaf, *viridis* and *vulgaris*, have been identified as comprising the plant material being grown in Cuba. The botanical descriptions presented show that the two varieties are alike in all respects in morphological appearance, with the exception of their leaves—*viridis* being characterized by having cordate leaves only, while *vulgaris* possesses both cordate and palmately lobed leaves. Differences in plant behavior within a particular variety suggest that these varieties are made up of several strains. These strains might offer a means of improving the plant for fiber purposes by selecting for one or two of the most desirable characters.

3. It is suggested that the differences in leaf shape are perhaps due to some ecological adaptation made during the evolution of the plant.

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CURRENT LITERATURE

John Torrey. By ANDREW DENNY RODGERS III. Princeton, New Jersey: Princeton University Press, 1942. Pp. 356. Illustrated. $3.75.

American Botany: 1873–1892. By ANDREW DENNY RODGERS III. Princeton, New Jersey: Princeton University Press, 1944. Pp. 350. Illustrated. $3.75.

These books, taken together, extend over approximately a century of development of botanical science in the United States, and for fuller understanding should be considered together. The one covers the life and work of JOHN TORREY. In it are developed details concerning TORREY's teaching, collections, travels, contributions, personal contacts and associations in founding systematic botany, and the establishment of two great herbaria and societies for scientific accomplishment in the United States. Glimpses are given of many of TORREY's contemporaries. Other than TORREY, the individual discussed

at greatest length and in detail is ASA GRAY, who collaborated extensively with him.

The second book, *American Botany*, takes up where the volume on JOHN TORREY leaves off. Now GRAY is made the central figure. In addition to his activities and contributions, those of HOOKER, ENGELMANN, PARRY, LESQUEREUX, GREENE, BRITTON, and many others are discussed, together with sketches on some of the younger men whose work extended for many years after the period covered by this book—1873–1892.

The results of many and varied exploring and collecting expeditions are given.

The author points out that ASA GRAY contributed much in addition to his work as a systematist. His outlook was philosophical, and he gave all scientific enquiry, particularly in the plant field, new impetus. He defended much of the work of CHARLES DARWIN and was conversant with the work of European scholars, both in botany and in horticulture. He was in no sense averse to the enlarging domain of botany into the areas which have become plant pathology, plant physiology, and the whole field of agricultural botany and scientific agriculture. In fact, he directly aided them. As a great teacher he prepared the way for advance through the training of students who took part in the newly and rapidly developing colleges of agriculture and the national experiment station movement.

The author tells something of the institutions concerned with the beginnings of this movement and the lives and work of some of the individuals active in it: ARTHUR, BAILEY, BEAL, BESSEY, BURRILL, HILGARD, and others. The founding of herbaria in addition to those of TORREY and of the United States National Herbarium, several botanic gardens, and several scientific organizations are noted. In fact, the number of individuals concerned with botanical investigations, the kinds and numbers of problems undertaken, and the places at which such work was carried out show so great and rapid increase during the decades discussed that the reader becomes all but lost in his attempts to follow them. Even so, a careful reading of this and the preceding volume affords rich reward to anyone who desires a reasonably clear background for an understanding of the evolution of the even more widespread work in the theoretical and applied botanical problems of the present.—E. J. KRAUS.

Root Disease Fungi: Vol. I. By S. D. GARRETT. Waltham, Mass.: Chronica Botanica Co.; New York City: G. E. Stechert & Co., 1944. Pp. 177. $4.50.

The appearance of this book marks the first volume of Annales Cryptogamici et Phytopathologici, edited by Frans Verdoorn. The author stresses the relationships between the inciting agents of these diseases and their habitats—the soil. The book is an interesting ecological study of an important group of causal agents within the larger field of root diseases of plants. The author has limited the scope of the presentation even more by not dealing "comprehensively either with all root-infecting fungi, or with the disease that they cause." Nevertheless, the book is a useful contribution to microbiological and phytoparasitological literature. The author concludes that the soil environment of microörganisms and plants varies less widely than the above-ground environment, especially in cultivated soils; that the problems of soil-disease control essentially are the same in tropical crops and in crops of the temperate regions; and that methods of control vary less with the region than with the type of cultivation, namely —"field, plantation, and glass-house cropping."

The first three chapters and chapters 7 and 8 are devoted to a consideration of the parasitism and saprophytism of root-infecting fungi. Chapters 8–15 concern themselves with control of root diseases incited by fungi.

The volume includes a bibliography, a general index, and an author's index. It is well written and the illustrations are excellent.—G. K. K. LINK.

A Manual of Soil Fungi. By JOSEPH C. GILMAN. Ames, Iowa: Collegiate Press, Inc., 1945. Pp. 392. Figs. 135. $5.00.

The purpose of this volume is to provide a tool in the identification of soil fungi. It includes chiefly those species which have been cultivated artificially on various types of biological media, excluding the terrestrial mushrooms; the plant pathogens (which though considered soil-borne have not been isolated directly from the soil); and the forms reported from leaf mold, decayed wood, or other substances not yet fully incorporated in the complex known as soil.

A brief introduction precedes the main body of the book, which consists of keys and species descriptions. The general keys are based on LINDAU'S System in ENGLER and PRANTL'S *Die natürlichen Pflanzenfamilien.* Species keys and descriptions are garnered from various pertinent monographs. More than half the pages are devoted to the Fungi Imperfecti, and all of these—save four—to the Moniliales. Of these 196 pages, 57 are taken up by 124 species of *Penicillium*, and 38 pages by 428 species and 24 varieties of *Fusarium*. The Saprolegniaceae occupy 61 pages, with the genus *Achlya* dominant with 25 species; the Mucorales require 47 pages, with the genus *Mucor* dominant with 42 species.

A bibliography, a glossary, and an index conclude the volume, which should prove useful to those interested in soil fungi.—G. K. K. LINK.